# THE REMOVAL OF INSECTICIDE RESIDUES FROM HARVESTED FRESH VEGETABLES

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By

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# INTRODUCTION

Vegetable crops were planted on 432,325 acres in Florida in 1952-53, and the gross Florida f.o.b. value of these crops was $154,320,000 (50). Vegetable production is a major industry of the state, and this status has been reached largely through continuing research on nutritional requirements, disease and insect control, and improved methods of marketing. Perhaps the most notable example of this progress has been the increase in the production of sweet corn from practically none in 1946 to more than 30,000 acres in 1953 with a value of more than $10,000,000. The principal contributing factor to this increase was the effectiveness of DDT as a control of corn earworms. Without the use of insecticides the vegetable industry of Florida would be seriously curtailed if not entirely eliminated.

Since the reporting of the insecticidal activity of dichlorodiphenyltrichloroethane (DDT) (32) and the discovery in Germany during World War II of the effectiveness of compounds of phosphorous acids against insects (38) as reported by Cassaday (7), many organic compounds have been synthesized specifically for their insecticidal properties. The chlorinated hydrocarbons, of which DDT, aldrin, toxaphene, and chlordane are representative, and the organic phosphatics, such as parathion, malathion, demeton, and TEPP, are the two classes of compounds that have been most widely exploited in this process. All of these compounds are included by the U. S. Food and Drug Administration in a list of substances being used on agricultural products and likely to leave residues that will create public health hazards in as much as they are toxic to warm-blooded animals (35,36).

To combat the problem of harmful residues, it is generally recommended that applications of these insecticides be stopped from 7 to 30 days before harvest (22). The basis for such a recommendation is the multitude of researches showing that some amount of the compounds remains on treated crops from 1 to 14 days or longer after application (9,13,25, 53,59). However, the insecticidal activity does not always persist for this length of time, and in Florida some of the more important insects may pass to a destructive stage in their life cycle in 7 days or less (42,45,63). Methods for the removal of any residue that might be present at the time of harvest would be of great benefit to the agriculture of Florida. Such removal would permit spraying to be continued nearer to the time of harvesting. Although treatment may not be necessary under present conditions, should increased insect resistance or other factors require additional applications, or an increase in the concentration of insecticides, it would be of the utmost importance to be able to suggest methods for the removal of excess residues.

The two main objectives of this project were to develop laboratory methods for the evaluation of the many washing materials available and to determine the effectiveness of materials thereby selected in removing residues from treated crops of celery, green beans, tomatoes, and mustard greens. Toxaphene and parathion were selected as being representative of the chlorinated hydrocarbons and organic phosphatics respectively.

## LITERATURE REVIEW

The amount of insecticide residues on vegetables at the time of harvest depends on several factors such as formulation of material, concentration, rate of application, growth habits and configuration of plants treated, length of time from application to harvest, and climatic factors during this period. Residues from insecticides have been a problem at least from the time arsenic sprays began to be used, but interest has been stimulated by the introduction of the highly potent and persistent newer materials.

### Persistence of Insecticides

Most of the information on the persistence of these insecticides has been reported as a part of studies on the insecticidal activity, and only a few are cited.

Decker et al (9) found parathion and lindane to be the least persistent of the materials tested, and emulsions of aldrin, chlordane, dieldrin, toxaphene, and DDT most persistent in that order. They reported residues of toxaphene of 50 ppm on peach foliage and 100 ppm on apple foliage 21 days after application of 1 pound per 100 gallons of spray. The resistance of toxaphene to removal by rain was greatest of all materials tested; however, toxaphene lost its insecticidal effectiveness more rapidly than it lost its ability to be detected by chemical analysis. The reported rate of loss was not uniform, but varied apparently in accord with the volatility of the compounds comprising the mixture since toxaphene is not a pure substance.

Vinson and Arant (59) applied toxaphene and parathion dusts to

peanuts, and after commercial harvesting for hay, found little if any parathion residue 7 and 35 days after application, but found 23 ppm toxaphene after 7 days and 4 ppm after 35 days.

Ginsberg et al (13) found 47 ppm of parathion on alfalfa immediately after dusting, but found the residue to be very low after 7 to 15 days, particularly after rains.

Stasch (53) reported a trace of toxaphene residue on celery 3 weeks after application of 10 per cent dust at a rate of 4 pounds per acre, and 28 ppm 3 weeks after application of 24 pounds per acre of a 60 per cent emulsion. She also reported that removal of outside leaves in the field and commercial washing and packing reduced the amount of residue remaining by about 40 per cent.

Three weeks after spraying, Stasch (53) found that toxaphene residues on green beans ranged from less than 0.5 ppm on samples from plots that had received 3 pounds per acre of toxaphene dust, wettable powder, or emulsion, to 5.5 to 12 ppm on those receiving 18 pounds per acre in an emulsion form. Toxaphene on tomatoes was found to be less than 5 ppm. She reported little effect of rainfall on residues of toxaphene.

Removal of Residues

Dehydration of alfalfa samples reduced the residues from toxaphene emulsions about 66 per cent from the original 81 ppm, and from parathion wettable powder about 84 per cent from the original 18 ppm, according to Stansbury and Dahm (54).

Gaines and Mistric (12) found that sprays of several insecticides were affected less by simulated rainfall than were dust formulations.

Toxaphene was not appreciably affected by rainfall although the emulsifier used had some slight effect on the persistence during the tests as well as on the toxicity of the insecticide.

Sloan et al (51) stated that DDT and parathion residues on Iceberg lettuce were shown to decrease 94 to 99 per cent during 2 weeks from the last application to harvest. Growth accounted for an approximate 73 per cent apparent decrease while the actual deposit loss by weathering was 80 and 99 per cent of the original for DDT and parathion respectively.

Residues from arsenical sprays generally were removed by the utilization of dilute solutions of hydrochloric acid. Schrader and Haller (49) reported in 1934 that the use of wetting agents in conjunction with acid was advantageous. Their tests showed that all wetting agents were not equally effective under all conditions.

Since the introduction of the newer organic insecticides, only a few papers devoted to the active removal of residues have appeared in the literature.

Manalo et al (37) treated apples individually with wettable DDT, and subjected them to polishing, peeling, brushing with a dry brush, and washing with various agents including naptha soap, Dreft, and organic solvents in dilute solutions emulsified with Dreft if necessary. Their washing apparatus consisted of a round brush mounted in a variable speed stirrer. The wash solution was placed in a small crock with the brush placed in the center. The apples were of a size to fit between the brush and the crock wall, and they rotated counter to the brush rotation, and traveled around the crock in the direction of the brush rotation. Their solution concentrations were from 0.1 per cent benzene to 1.0 per cent

soap and 1.0 per cent acetone. The washing period was 15 minutes for all solutions except that it was 10 minutes for the organic solvents. A 5- to 10-minute rinsing period in tap water in the same apparatus followed each washing. Only peeling removed all the DDT residues; polishing removed 23 to 33 per cent; brushing removed 36 per cent; and washing treatments removed 10 to 41 per cent. None of the washing solutions consistently removed more residue than tap water alone. They attributed all removal by the washings to mechanical dislodging.

Walker (60) reported variable results with detergent materials for removing DDT residues from apples and pears, but concluded that none of the wash treatments was effective.

Laudani and Marzke (31) treated wool cloth squares with 0.5, 1.0, 1.5, 2.0, and 3.0 per cent solutions of insecticides including toxaphene, and allowed them to dry for 7 days before subjecting them to washing and dry cleaning. They were washed for 30 minutes at 42 rpm in a solution of 5 grams of soap per liter, or dry-cleaned for 20 minutes in Stoddard solvent. Mortality to fabric insects feeding on the cloth was used as an index of persistence. Complete mortality occurred after one washing of higher toxaphene concentrations, and limited protection from insect damage remained after the third washing. Some mortality to insects also occurred after the third dry cleaning.

Gunther et al (18) studied the removal of DDT and parathion residues from the surfaces of apples, pears, lemons, and oranges. They were successful in reducing residues of 3.3 to 9.5 ppm of DDT by 82 to 98 per cent with a number of materials: sodium silicate (70 to 82 pounds of 58.5 Baume' solution per 100 gallons of water), 1.25 per cent hydrochloric acid, soap (4 pounds per 100 gallons), and trisodium phosphate (4 pounds

per 100 gallons). Sodium silicate frequently proved superior for the removal of residues of wettable DDT from apples and pears, and soap removed significant amounts from oranges. Parathion residues were not significantly reduced by any treatment. Apples and pears were washed by standard packinghouse techniques in which the fruit was exposed to the wash solutions 25 to 30 seconds, rinsed, and passed through a roller dryer. Oranges and lemons were also subjected to normal packinghouse treatments which included a thorough machine-scrubbing. No data were presented to show what reduction of residues would have occurred had water alone been used in these commercial processes. The failure to remove parathion was attributed to the apparent solubility of parathion in many plant oils and waxes.

McBain and Richards (39) reported on the solubilization of insoluble liquids by detergents, and postulated that any substance can be made soluble in any medium by the use of the proper solubilizing agent. They used a number of cation-active and anion-active detergents on a series of aliphatic and aromatic hydrocarbons and a number of polar compounds. In general, the various detergents showed parallel behavior, merely differing in degree of solubilizing power. Cation-active agents were generally more effective. There were, however, numerous specificities and influences of structure, both of the detergent and of the material being solubilized.

McCutcheon (40,41) has a list of several hundred synthetic detergents made by 117 companies. This list gives the name, formula, main uses, and some properties of the compounds. In addition to this list, most manufacturers provide detailed information and suggestions

for the use of their materials.

## Toxicity of Insecticides

The toxicity of toxaphene to warm blooded animals has been well established by Lackey (28), Lehman (33,34), and the Agricultural Research Administration of the USDA (57). Lehman (33), however, reported that rats could ingest a quantity of the compound in the course of 24 hours which would have been fatal if administered as a single dose. It is known that camphor, to which toxaphene bears a close chemical relationship, is readily detoxified in the liver, and he believed that when toxaphene is ingested in small quantities over a period of time the detoxification keeps pace with the intake.

Data presented in Docket FDC-57 of the Federal Security Agency[1] (44), and that reported by Hazleton and Holland (24) and by Lehman (33,34) clearly demonstrate the toxicity of parathion. The toxicology of the material and precautions to be observed in its use in Florida have been presented by Griffiths et al (16).

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[1]This agency became the Department of Health, Education and Welfare in 1953.

## MATERIALS AND METHODS

Celery, Apium graveolens, Linn.; green beans, Phaseolus vulgaris, (Tourn.) L.; tomatoes, Lycopersicon esculentum, Mill.; and mustard greens, Brassica juncea, Coss., were chosen as representative of four general types of vegetables that are of importance in Florida agriculture. Toxaphene and parathion were selected as the insecticides to be used for reasons discussed below. It should not be construed that this is a recommendation for the use of these materials on those crops, although they are effective against many insects attacking them.

### Spray Materials

#### Toxaphene

Toxaphene was selected as one of the insecticides used in this study because: (1) it is a good example of the chlorinated hydrocarbon insecticides, (2) its persistence as an effective residual spray is prolonged, particularly when applied as an emulsion spray, (3) it is effective against a number of important insect pests, and (4) of the insecticides for which such data have been reported, it is apparently the most resistant to the removal effects of water.

The material is formed by the chlorination of camphene to a chlorine content of 67 to 69 per cent, and has the empirical formula $C_{10}H_{10}Cl_8$. The exact formula is unknown, and the technical product, according to Decker et al (2) is not a pure substance but a mixture of compounds. It is a light yellow, waxy material with a slight piny odor and a melting point range of 65° to 90°C. It is very soluble in most of the common organic solvents.

The major disadvantage to its use in residue studies is that a specific method of analysis has not been available. The methods used depend upon the determination of the total amount of chlorine present and calculation of toxaphene based on the minimum chlorine content of technical toxaphene of 67 per cent. The procedures used in this study generally followed the method adapted by the Hercules Powder Company (56) and used for residue work by the Florida Agricultural Experiment Station since the material was introduced. It is presented in detail in Appendix A. In general, the insecticide in isopropanol solution was decomposed by sodium, and the resulting chlorides determined by argentometric amperometric titration using a rotating platinum microelectrode. Obviously chlorine from any extraneous source will be determined and erroneously calculated as toxaphene. However, in the absence of other chlorine containing insecticides, and with proper blank determinations, Carter et al (6) were able to show close agreement between bioassay and total chlorine methods of analysis of toxaphene.

An emulsion formulation by California Spray Chemical Corporation containing 8 pounds of technical toxaphene per gallon was used in all phases of this study, except that celery was sprayed with an emulsion from the same source containing 9.6 pounds of toxaphene per gallon.

Parathion

Parathion is perhaps the best known of the organic phosphate insecticides. It has been widely studied in its mode of action, translocation, and persistence. It is the only one of the phosphatics for which the author was able to find any information in the literature pertaining to the removal of residues.

Parathion is the accepted trivial name of O,O-diethyl,O-p-nitrophenyl thiophosphate, a pale yellow, practically odorless oil which crystallizes in long, white needles at 6.0°C., according to Hall (19). It is soluble to the extent of 15 to 20 ppm in water, and is soluble in organic solvents except the low aromatic kerosenes.

A specific, highly sensitive method of analysis is available (2). This method, in general, consists of reduction of the nitro group to the amine, diazotization, and coupling with a dye intermediate to form an intense magenta color which can be quantitatively measured with spectrophotometric apparatus. This method, with modifications, was used in this work, and details of the procedures are given in Appendix A.

An emulsion formulation by Florida Agricultural Supply Company containing 2 pounds of parathion per gallon was used throughout this work.

## Spray Equipment

The sprayer used for applying insecticides to celery was tractor mounted and covered four rows at a passage. Hollow-cone nozzles were arranged, five per row, on the boom in such a way that a single nozzle directed spray straight down on top of the row, two nozzles were directed downward from the top and sides at an angle of 45°, and two nozzles were directed upward from the bottom and sides at an angle of 15°. With the tractor driven at 4 miles per hour, about 125 gallons of spray per acre were applied at a pump pressure of approximately 300 pounds per square inch.

Spray was applied to all other plots with a 3-gallon, compressed-air hand sprayer. This sprayer was fitted near the bottom with a rapid-coupling inlet valve to permit the attachment of an air hose from a

portable compressed-air tank. The hose was long enough to permit the sprayer to be used in small plots with pressure being maintained continuously. It was found to be more expeditious, however, to fill the tank to the desired maximum pressure, disconnect the hose, spray until pressure dropped to a desired minimum, and then refill to the maximum pressure. Refilling with air kept the spray well mixed by air passage through the solution. Pressure was maintained at 35 to 45 pounds during spraying. The sprayer head was made so that 1, 2, or 3 hollow-cone nozzles could be used depending on the size of the crop (Fig. 1).

### Detergents

Several of the leading detergent manufacturing companies were asked for recommendations for the use of any detergent materials made by them which they thought might be applicable to the problem of residue removal. The materials generously contributed by them were catalogued as to composition, and fifteen were selected as specific compounds or as representative of a general class of compounds (Table 1). In the event two or more compounds were substantially the same, the test material was randomly selected.

### Other Equipment

For purposes of clarity and coherence, details of washers used for the various crops, extracting equipment, and procedures followed are presented, along with the results of preliminary, screening, and field tests, as a part of the section on experimental work which follows.

Fig. 1 — Compressed-air sprayer used for applying insecticides to test plots.

Note the auxillary compressed-air tank and the interchangeable nozzle arrangement.

Table 1.—Name, composition, and manufacturer of detergents tested for their effectiveness in removing insecticide residues from fresh vegetables.

| Name | Composition | Manufacturer |
|---|---|---|
| 1. Orvus neutral granules | Sodium salt of technical lauryl sulfate | Proctor and Gamble |
| 2. Olate flakes | Neutral soap | Proctor and Gamble |
| 3. Triton X-100 | Alkyl-aryl polyether alcohol | Rohm and Haas |
| 4. Nytron | Sulfonated ketones, amines, alkyl sulfamates, alkylidene sulfamates, alkylidene sulfamate bisulfite | Solvay Sales Corporation |
| 5. Mentor beads | Alkyl-aryl sulfonate, 33% | Colgate-Palmolive-Peet Company |
| 6. Arctic Syntex HD | Alkyl-aryl sulfonate plus builders [a] | Colgate-Palmolive-Peet Company |
| 7. Emulsifier "M" | Blend of alkyl-aryl sulfonate and polyoxyethylene ester | Monsanto Chemical Company |
| 8. Tergitol 7 | Sodium heptadecyl sulfate | Carbide and Carbon Chemical Company |
| 9. Tergitol NPX | Alkyl-aryl polyglycol ethers | Carbide and Carbon Chemical Company |
| 10. Vatsol OTB | Sodium dioctyl sulfo-succinate | American Cyanamid Company |
| 11. G 8916 T | Polyoxyethylene sorbitan esters of mixed fatty and resin acids | Atlas Powder Company |
| 12. Myrj 53 | Polyoxyethylene stearate | Atlas Powder Company |

[a] Builders are such materials as the poly- and pyrophosphates added to the base detergent to aid in the suspension of the material.

Table 1.—Continued

| Name | Composition | Manufacturer |
|---|---|---|
| 13. Brij 35 | Polyoxyethylene lauryl alcohol | Atlas Powder Company |
| 14. Tween 20 | Polyoxyethylene sorbitan monostearate | Atlas Powder Company |
| 15. G 2162 | Polyoxyethylene propylene glycol monostearate | Atlas Powder Company |

# EXPERIMENTAL

## EXPERIMENTAL

One of the difficulties that has been encountered in residue work is the variability of samples due to the biological differences of treated crops. For example, tomatoes may come from plants with heavy or sparse foliage or celery plants may be loose or compact. Large quantities of produce are therefore necessary to provide enough samples of sufficient size to minimize the effects of this variability. Thus it would seem impractical to use treated produce to determine which of many hundred detergent materials is most effective in removing insecticide residues, particularly when differences in effectiveness may be slight.

Data presented to the Food and Drug Administration by the American Cyanamid Company (44) indicated that the loss of parathion from treated filter paper was similar to its loss from treated plants.

### Preliminary Tests

The experimental work began with preliminary tests to determine whether treated filter papers could be washed in laboratory apparatus and this simplified procedure used to screen the numerous detergents. Whatman #50 filter paper is a very tough paper, specially hardened by treatment with nitric acid, resists great pressure, retains very fine precipitates, and can be repeatedly washed without injury to the paper. Nine-centimeter papers were found to require about 0.5 ml. of toxaphene or parathion emulsion for saturation, and concentrations were made so that each paper contained 20 to 25 mg. of toxaphene or 5 to 10 mg. of parathion after treating and air drying.

The papers were held in position in the washing apparatus (Fig. 2) by clamps holding the paper practically immobile. The papers were washed in a water spray or immersed in a tank of gently circulating water. Spray was made and distributed over both surfaces of the paper by fog nozzles operated at 35 pounds pressure. By the use of fog nozzles or water moving only slightly in the tank, mechanical pressure forces were kept at a minimum to give a better per se evaluation of the wash solution being used. Results given in Table 2 show the effect of tap water applied by fog nozzles on the residues, and Table 3 indicates the effect of a water-immersion wash.

Table 2.—Toxaphene and parathion in mg. on treated filter papers water-washed with fog nozzles.[a]

| Insecticide applied | Washing time | | |
|---|---|---|---|
| | 0 Minutes | 5 Minutes | 15 Minutes |
| Toxaphene | 19.83 | 19.34 | 19.06 |
| Parathion | 10.27 | 10.04 | 9.93 |

Insecticide mean differences are non-significant.

[a] Each value is the mean of duplicate determinations of three replicates.

Fig. 2 - - Apparatus designed for washing treated filter papers.

Water entering through the hose could be used for washing or rinsing. Solutions from an auxiliary tank could also be pumped through the nozzles.

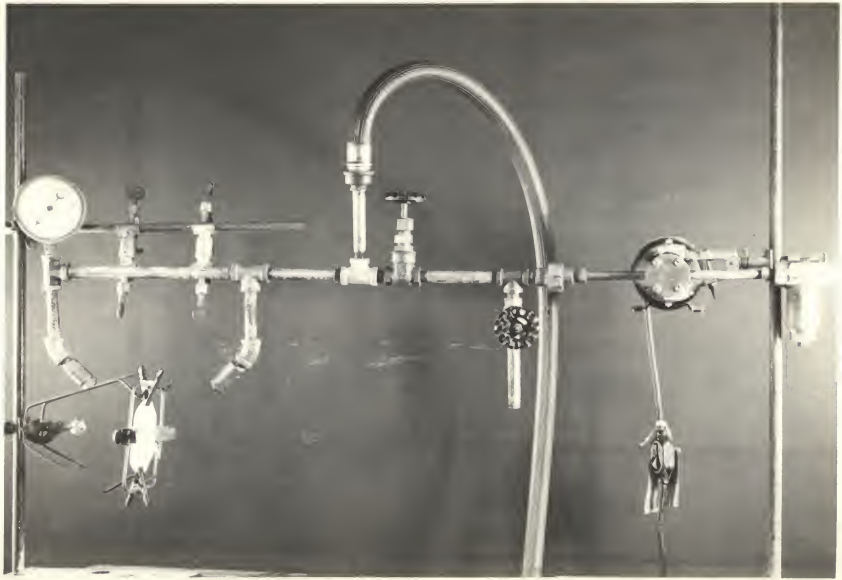

Table 3.—Toxaphene and parathion in mg. on treated filter papers water-washed by immersion. [a]

| Insecticide applied | Washing time | | |
|---|---|---|---|
| | 0 Minutes | 5 Minutes | 15 Minutes |
| Toxaphene | 22.85 | 22.35 | 21.92 |
| Parathion | 9.33 | 9.12 | 9.02 |

Insecticide mean differences are non-significant.

[a] Each value is the mean of duplicate determinations of three replicates.

As a result of these preliminary tests, it appeared that water alone with a minimum of agitation or force did not remove appreciable amounts of either of the test insecticides, and, therefore, screening tests could be established in which any significant reduction in the amount of insecticides on the filter papers should be due to the action of the detergent.

## Screening Tests

All detergent samples were catalogued according to composition, and fifteen were selected as specific compounds or representative of a general class of compounds. These were randomly assigned to groups of three to facilitate the analysis which was conducted in units of twelve samples. Thus three replicate samples of three detergents and a water-wash check constituted each 12-sample group.

On the basis of results of preliminary tests, a 10-minute washing by immersion followed by a rinsing period of 2 minutes under a water spray was established as the standard to be followed in the screening tests. Primary consideration was given to this type of washing procedure since it seemed to be the type that would be most adaptable for packing-house use (Fig. 3). It was also the opinion that a washing time of 10 minutes would be adequate to permit treatment differences that might exist to be evaluated properly. The 2-minute rinsing period allowed for the apparent removal of detergents remaining on the papers after washing.

Since toxaphene is more resistant than parathion to removal by water (rainfall), this insecticide was used for the majority of the screening tests. Those detergents which removed the highest percentage of toxaphene from the treated papers were further evaluated by using papers treated with parathion.

Twelve 9-cm. Whatman #50 filter papers were uniformly saturated with 0.5 ml. of an emulsion containing 40 to 45 mg. of toxaphene or 5 to 10 mg. of parathion per milliliter. The papers were allowed to dry at room temperatures (70° to 90°F.), and then were washed in a random order. The papers were clamped in the holders and immersed in a horizontal position (for convenience only) in the wash solutions in such a way that the solution circulation was not impaired on either surface. The solutions were agitated by a propellor-type mixer only enough to create a constant circulation. Detergent concentration was based wherever possible on the manufacturer's recommendation; otherwise, it was based on available information for that or a similar compound. The wash solution was removed from the tank after each paper had been washed, and the apparatus

Fig. 3 - - Commercial Washing of Celery

was rinsed. However, all three papers of any one treatment were washed in the same solution. No apparent differences in replicates due to this were noted, and it was felt that any satisfactory washing material should be able to maintain its effectiveness for several washings.

After washing for 10 minutes, the papers, in their holders, were rinsed for 2 minutes in a spray of tap water from the fog nozzles. The papers were then air-dried, and the residue remaining extracted by placing the papers in 100 ml. of benzene in a closed jar and allowing overnight extraction. A shorter extraction time perhaps would have been adequate, but it was generally convenient to allow overnight standing for this step. Aliquots of this extract solution were used for residue analyses by the indicated methods. Results are given in Table 4.

These tests were repeated, in part, using toxaphene concentrations of 75 to 100 mg. per paper, and results were generally the same.

From these screening tests, it was apparent that wide differences in the effectiveness of detergents in removing residues occurred. The differences in magnitude and order of effectiveness between insecticides were not great, and, in the light of later work, probably were due to sampling variation.

These results indicated that this laboratory method was an effective tool for the screening of large numbers of washing materials to determine which were most effective in their removal action on residues of toxaphene and parathion.

Table 4.—Toxaphene and parathion residues on treated filter papers following detergent washing.

| Washing solution | | Insecticide (mg) [a] | | Per cent less than after water washing | |
|---|---|---|---|---|---|
| Name | Conc. | Toxaphene | Parathion | Toxaphene | Parathion |
| | % | | | | |
| Water | - - - | 25.20 | 10.53 | - - - | - - - |
| Triton X-100 | 1.0 [b] | 18.69 | 8.41 | 25.83 | 20.13 |
| Olate flakes | 1.0 | 19.12 | 8.55 | 24.13 | 18.80 |
| Tween 20 | 1.0 | 22.14 | - - - | 12.14 | - - - |
| Water | - - - | 21.49 | - - - | - - - | - - - |
| Arctic Syntex HD | 0.5 | 19.39 | - - - | 9.77 | - - - |
| Tergitol 7 | 1.0 | 19.34 | - - - | 10.01 | - - - |
| Myrj 53 | 0.5 | 21.17 | - - - | 1.49 | - - - |
| Water | - - - | 22.86 | - - - | - - - | - - - |
| Emulsifier "M" | 0.5 [b] | 18.67 | 8.10 | 18.33 | 23.08 |
| Tergitol NPX | 1.0 | 18.50 | - - - | 19.08 | - - - |
| G 2162 | 0.5 | 19.14 | - - - | 16.27 | - - - |
| Water | - - - | 21.56 | - - - | - - - | - - - |
| Orvus neutral | 0.5 | 19.41 | - - - | 9.97 | - - - |
| Mentor beads | 0.5 | 20.41 | - - - | 5.29 | - - - |
| Brij 35 | 0.5 | 19.76 | - - - | 8.35 | - - - |
| Water | - - - | 24.96 | - - - | - - - | - - - |
| Nytron | 0.2 | 23.97 | - - - | 3.97 | - - - |
| Vatsol OTB | 0.1 | 22.33 | - - - | 10.54 | - - - |
| G 8916 T | 0.5 | 20.78 | - - - | 16.75 | - - - |

[a] Each value is the mean of duplicate determinations of three replicates.

[b] Concentrations reduced to 0.1 per cent for parathion and later tests.

Removal of Insecticides from Treated Vegetables

Of the fifteen detergents evaluated in screening tests, only four were considered effective enough for the removal of insecticide residues to warrant their use on treated vegetables. These four materials were: (1) Triton X-100, (2) Olate flakes, (3) Tergitol NPX, and (4) Emulsifier "M". Celery, green beans, tomatoes, and mustard greens were selected as representative vegetables for these tests.

Celery - - Winter 1952-53

The first of these tests was made on celery grown at the Central Florida Experiment Station in Sanford. Florida Pascal variety of celery was transplanted to the field on November 19, 1952, into six blocks, each 30 feet long by four 30-inch rows wide. Blocks were separated at the ends by a 12-foot space. Normal cultural practices were followed except that no insecticides other than toxaphene were applied, and it was not necessary to use any fungicides. California Spray Chemical Corporation "All-Tox" containing 9.6 pounds of toxaphene per gallon was applied at a rate of 2.5 pints per 100 gallons per acre, with 6 ounces of Triton B-1956 per 100 gallons used as a spreader-sticker. Applications were made with a tractor-mounted sprayer. The first application of toxaphene was made on January 27, and subsequent applications were made at weekly intervals through February 17. Harvesting was done on February 17 and March 3. Minimum air temperatures from January 27 through March 3 were from 38° to 62°F. and averaged 51°; maximums were from 61° to 88°F. and averaged 75°. Total rainfall during this period was 2.97 inches: 0.93 inch the first 2 days after the second application of spray, 0.56 inch 2 days before the third application, 0.60 inch 2 days prior to the

fourth application, and 0.84 inch during the 2-weeks period between harvests. Unmeasured traces fell at other times.

The alternate ends of the two center rows were harvested on February 17, 2 hours after the fourth application of spray. Approximately 100 stalks[2] were cut from each block, trimmed to a standard 18-inch length, and packed in two wirebound celery crates. Two crates of plants were also cut from check plots that had received no spray. All of the celery was placed in cold storage in Gainesville about 6 hours after harvesting. Storage temperature was 40°F., and relative humidity was 90 to 100 per cent.

Washing treatments and residue extraction of samples began 43 hours after harvesting. Block 1 was treated and residues extracted the first day, Block 2 the second day, until Block 6 was treated on the sixth day. This procedure was followed because of the physical impossibility of completing the washing and extraction of more than one block in any one day.

The celery from each block was mixed and divided into five lots of 18 stalks each, and a sixth lot of the same size was taken from the two crates of the check plots. Treatments were assigned to each lot in a random manner, and washing treatments were made in a random order. Treatments were:(A) unsprayed celery washed in water, (B) sprayed celery that was not washed, (C) sprayed celery washed in water, (D) sprayed celery washed in a 1.0 per cent solution of Triton X-100 (following a

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[2] Stalk as used herein refers to the entire plant minus roots and leaves removed in the field.

conference with the supplier, this concentration was reduced to 0.1 per cent for all subsequent tests), (E) sprayed celery washed in a 1.0 per cent solution of Olate flakes, and (F) sprayed celery washed in a 1.0 per cent solution of Tergitol NPX (this concentration was also reduced to 0.1 per cent in later tests in an effort to reduce excessive weight losses of the celery). All solutions were calculated as per cent by weight, and temperatures were kept at about 72°F.

The 18 stalks of celery in each treatment were placed in a tank containing 30 liters of the treatment solution and allowed to remain for 10 minutes. The wash solution was continuously circulated over and through the stalks by a centrifugal pump with a bottom inlet and a top of the tank outlet. Solutions were discarded and the equipment thoroughly rinsed after each treatment. After 10 minutes washing, the celery was transferred to a spray-washer for rinsing. This washer consisted of eight side and four top "Fulljet" nozzles delivering 1.5 to 2.0 gallons of water per minute at a pressure of 15 to 20 pounds (Fig. 4). The speed of the wire-mesh conveyor belt could not be reduced sufficiently to allow for thorough rinsing in one passage, therefore, in order to provide most efficient usage of the spray and to simulate a possible commercial assembly, the celery was run through repeatedly until thoroughly rinsed. To standardize procedure, the celery was spaced on the belt in such a way that complete rinsing was accomplished in three passages through the washer. The celery was then placed in wooden racks and allowed to drain for about 2 hours before the residue was extracted. Even though some difficulty had been encountered in extraction of wet filter papers, a preliminary test showed no appreciable difference in

Fig. 4 - - Spray-washer used in the laboratory for rinsing vegetables after detergent washing.

the amount of residue extracted from wet and dry celery. The draining period, therefore, was a matter of convenience in the procedural routine as well as a precautionary safeguard and a way to remove most of the free moisture from the celery before weighing samples.

Quality

Six stalks of each of the sprayed and washed treatments were weighed and placed together in a wirebound crate and stored at 40°F. and 90 to 100 per cent relative humidity. They were weighed and examined at 2-day intervals for 6 days to determine the effects of the detergents on the celery quality as measured by injury, weight loss, flavor, color, and general appearance.

The celery in all washed treatments showed some indications of water-soaking immediately after washing, but the affected areas had regained their normal appearance at the time of weighing and storage. Celery washed in detergent solutions had a very good, clean appearance after washing, but was not enough different from the water-wash treatment to be distinguished by casual observation. Some soil particles remained in the center of the stalks of all treatments. The detergents had no apparent effect on color, flavor, or general appearance, and they produced no indications of injury at the concentrations used. Differences in weight loss did occur, and per cent losses are shown in Table 5.

An analysis of variance revealed that most of the differences in weight loss observed could be attributed to the use of Tergitol NPI, although by the sixth day all of the detergent samples had lost significantly more weight than those washed only in water.

Table 5.- -The effect of detergent washing on the weight loss of celery during storage at 40°F. - - Winter 1952-53.

| Days storage | Weight loss in per cent | | | |
|---|---|---|---|---|
| | Washing treatment | | | |
| | Water | Triton X-100 | Olate flakes | Tergitol NPX |
| | Harvest I - 2 hours after spraying [a] | | | |
| 2 | 2.67 | 2.55 | 2.78 | 3.98 |
| 4 | 3.75 | 4.34 | 4.12 | 5.47 |
| 6 | 4.60 | 5.47 | 5.23 | 6.49 |
| | Harvest II - 2 weeks after spraying [b] | | | |
| 2 | 2.33 | 2.46 | 2.84 | 2.60 |
| 4 | 3.49 | 3.38 | 3.74 | 3.46 |
| 6 | 4.29 | 4.10 | 4.77 | 4.47 |

L.S.D. for treatment means within storage days of Harvest I - -0.05 = 0.51

0.01 = 0.71

Detergent mean differences of Harvest II are non-significant.

[a] Each value is the mean of six replications.

[b] Each value is the mean of five replications.

The remaining celery of the two center rows of each block was harvested on March 3, and subsequent treatment was the same as above, except the concentrations of Triton X-100 and Tergitol NPX were reduced to 0.1 per cent in an attempt to reduce the amount of weight loss during storage.

Results of quality tests were, in general, the same except that overall weight loss was slightly less, probably due to the maturity of the celery (Table 5). Weight losses of both harvests are shown graphically in Fig. 5. The effects of reducing the concentrations of Triton X-100 and Tergitol NPX were such that, combined with other factors such as celery maturity, there were no significant effects of detergents on the weight loss of celery of the second harvest.

Residues

Twelve stalks of each treatment were divided into top, or leaf blade, and bottom, or petiole, portions (Fig. 6). This division was essentially to separate the blade of the leaf from the petiole. The separation was not complete, but only a few pieces of the petiole were in blade portions and vice versa. Although this procedure may be a possible source of error, it is the standard that has been generally adopted for residue work on this crop. Each of these portions was cut into 1- or 2-inch pieces, thoroughly mixed, and 500 gram leaf blade samples and 1000 gram petiole samples taken for extraction. The sample portions were placed in 1-gallon wide-mouth jars, 1000 ml. of benzene added, the tops tightly fastened (with two to four layers of MSAT cellophane to prevent contamination of the top gasket), and the surface residue extracted by rotating the jars for 30 minutes at a speed of 32 rpm. (Fig. 7). After extraction, the water layer was allowed to settle, the benzene was decanted, and 400 ml. aliquots of the benzene solution placed in pint jars and stored at 30° to 40°F. Toxaphene analyses were made by the indicated method.

Fig. 5 — The effect of detergent washing on the weight loss of celery during storage at 40°F.

Subscript numbers refer to harvests. Letters refer to washing solutions: "C" refers to water, "D" to Triton X-100, "E" to Olate flakes, and "F" to Tergitol NPX.

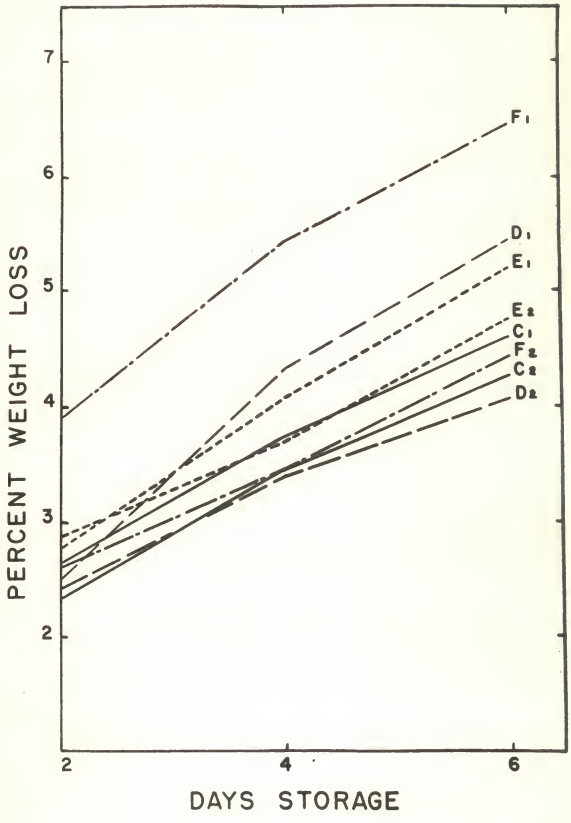

PERCENT WEIGHT LOSS
7
6
5
4
3
2
2
4
6
DAYS STORAGE
F1
D1
E1
E2
C1
F2
C2
D2

Fig. 6 - - Celery divided into leaf blade and petiole portions prior to residue extraction.

Fig. 7 - - Tumbling Apparatus for Residue Extraction

(Photograph courtesy of G. H. Blackmon)

The results given in Tables 6 and 7 show the parts per million (milligrams per 1000 grams of celery) of toxaphene. No tests of statistical significance were applied to these data because of the numerous difficulties encountered during the analyses and indicated in more detail in Appendix A. Data in the tables marked "corrected data," have been corrected because of the occurrence of a relatively constant error during some titrations of the samples of the second harvest. This error appeared to be related to the functioning of the amperometric titration assembly and may have been caused by a cracked electrode. It was quite apparent when the discrepancy occurred, and the correction is believed to be a valid one.

The amount of residue shown for the check treatment was great in all cases and was quite variable. These high values are believed to be due to the numerous causes of error such as contaminated benzene, a malfunctioning central water still, and unknown interfering substances that became evident as the work progressed. However, by applying the average check value to the corrected data, the resulting parts per million of toxaphene were not appreciably different from results obtained by other workers (53) for toxaphene residues on celery from the same location. No conclusions, however, can be confidently based on these results, and they are included only for general comparison with results on other crops.

Table 6. - - Toxaphene residues on celery leaf blades following detergent washing. - - Winter 1952-53.

| Block | Toxaphene in mg. per 1000 grams of celery (ppm) [a] | | | | | |
|---|---|---|---|---|---|---|
| | Washing treatment | | | | | |
| | Check | None | Water | Triton X-100 | Olate flakes | Tergitol NPX |
| | Harvest I - 2 hours after spraying | | | | | |
| 1 | 31.51 | 64.48 | 44.58 | 78.91 | 44.80 | 69.09 |
| 2 | 24.58 | 107.41 | 36.39 | 48.57 | 54.60 | 50.26 |
| 3 | 32.78 | 116.82 | 120.23 | 58.66 | 81.70 | 85.62 |
| 5 | 52.88 | 263.77 | 106.19 | 59.43 | 73.85 | 74.29 |
| 6 | 66.37 | 191.08 | 132.88 | 88.78 | 71.23 | 85.34 |
| Treatment mean | 41.62 | 148.71 | 88.05 | 66.87 | 65.24 | 72.92 |
| | Harvest II - 2 weeks after spraying | | | | | |
| 1 | 30.20 | 33.08 | 45.40 | 48.78 | 32.18 | 41.77 |
| 2 | 23.04 | 44.64 | 39.96 | 23.91 | 35.57 | 24.25 |
| 3 [b] | 44.56 | 40.72 | 72.40 | - - - | 46.08 | 96.96 |
| 4 | - - - | 67.94 | 63.70 | 50.16 | 71.59 | 46.28 |
| 5 [b] | 59.28 | 51.10 | 56.20 | 62.36 | 27.32 | 66.16 |
| Treatment mean | 39.27 | 55.65 | 55.53 | 46.30 | 42.55 | 55.08 |

[a] Each value is the mean of duplicate determinations of duplicate samples.

[b] Corrected data.

Table 7. - - Toxaphene residues on celery petioles following detergent washing. - - Winter 1952-53.

| Block | Toxaphene in mg. per 1000 grams of celery (ppm) [a] | | | | | |
|---|---|---|---|---|---|---|
| | Washing Treatment | | | | | |
| | Check | None | Water | Triton X-100 | Olate flakes | Tergitol NPX |
| | Harvest I - 2 hours after spraying | | | | | |
| 1 | 14.36 | 16.53 | 19.95 | 36.78 | 22.39 | 22.04 |
| 2 | 16.18 | 37.48 | 21.26 | 24.23 | 29.23 | 39.85 |
| 3 | 23.51 | 30.91 | 20.61 | 19.08 | 38.16 | 19.08 |
| 5 | 14.64 | 45.32 | 21.41 | 25.35 | 20.10 | 21.19 |
| 6 | 18.59 | 26.08 | 30.05 | 25.51 | 23.52 | 17.92 |
| Treatment mean | 17.46 | 31.26 | 22.66 | 26.19 | 26.68 | 24.02 |
| | Harvest II - 2 weeks after spraying | | | | | |
| 1 | 8.20 | 21.66 | 17.82 | 22.28 | 20.46 | 15.24 |
| 2 | 11.52 | 16.13 | 16.27 | 15.48 | 12.65 | 13.76 |
| 3 [b] | 18.40 | 31.62 | 25.31 | 25.92 | 29.87 | 28.66 |
| 4 | 27.82 | 26.90 | 23.94 | 25.42 | 21.20 | 20.62 |
| 5 [b] | 26.10 | 21.32 | 23.82 | 33.18 | 28.56 | 21.60 |
| Treatment mean | 18.40 | 23.53 | 21.43 | 24.46 | 22.55 | 19.98 |

[a] Each value is the mean of duplicate determinations of duplicate samples.

[b] Corrected data.

Green Beans - - Spring, 1953.

Contender variety of green beans was planted in Gainesville on March 5, 1953, in six replicated blocks, each 25 feet long and eight rows wide. Rows were spaced 34 inches apart, and the row most distant from power spraying in the direction of prevailing winds was designated as a check row and received no spray. It was separated from treated rows by guard rows. Normal cultural practices, other than spraying, were followed. The plants became very heavily infected with root-rot organisms such as Pythium sp. and Rhizoctonia sp., and stands became spotty, plant vigor poor, and yields low. Therefore, to obtain adequate harvests, adjacent plots were combined leaving only three replicates.

Spray materials were applied with the compressed-air sprayer previously described. Toxaphene was used at a rate of 2.5 pounds of active material per 100 gallons per acre, and was made from an emulsion formulation containing 8.0 pounds technical toxaphene per gallon. Parathion was used at a rate of 0.5 pound active material per 100 gallons per acre, and was made from a 25 per cent emulsion formulation. Triton B-1956 was used at a concentration of 4 ounces per 100 gallons as a spreader-sticker. All blocks were uniformly sprayed on April 28 as a specific control measure. The first replicate only was sprayed again on May 5. Twenty-four hours later on May 6, the first replicate was harvested, and the second replicate sprayed. The second replicate was harvested the following day, and the third sprayed. The third replicate then was harvested on May 8. This procedure was followed in order to facilitate spraying, and, particularly, so that washing and residue extraction in the laboratory could be completed the same day that the

beans were harvested. A heavy rain of 1.4 inches fell between the spraying and harvesting of the second replication. Air temperatures in the field during the time the spray was on the plants were from 60° to 90°F. and averaged 75°F.

Approximately 4 pounds of beans from each sprayed row were mixed together and divided into five lots of about 3.5 pounds each. The same quantity of beans from the unsprayed check row constituted the sixth lot. The beans of each lot were placed in a rectangular basket of one-fourth inch hardware cloth and washed. Washing and rinsing procedures were the same as for celery, i.e., 10 minutes washing followed by a thorough rinsing and a 2-hour draining period. The washing treatments were: (A) unsprayed beans washed in water, (B) sprayed beans not washed, (C) sprayed beans washed in water, (D) sprayed beans washed in a 0.1 per cent solution of Triton X-100, (E) sprayed beans washed in a 1.0 per cent solution of Olate flakes, and (G) sprayed beans washed in a 0.1 per cent solution of Emulsifier "M". All solutions were calculated as per cent by weight, and temperatures were kept at about 70°F. Assignment of treatments and order of washing were completely randomized.

Quality

A 500 gram sample from each of the sprayed and washed treatments was taken from the washed beans and stored in wire-mesh baskets at 40°F. and 90 to 100 per cent relative humidity. Samples were weighed and examined at 2-day intervals for 6 days. Quality evaluation was based on weight loss, color, injury, flavor, odor, and general appearance.

The beans washed in Triton X-100 and Emulsifier "M" showed indications of apparent injury after 4 days storage. This injury appeared

as small, slightly sunken, discolored spots on the beans. The beans washed in water or in a solution of Olate flakes were only slightly discolored with no injury apparent after 6 days storage. Six days storage resulted in tough and flaccid beans in all treatments, and, combined with the very high loss of weight, indicated generally unsatisfactory storage conditions. There were no apparent effects of detergents on flavor, color, or odor. Weight losses (Table 8) were quite great even after 2 days storage.

Table 8. -- The effect of detergent washing on the weight loss of green beans during storage at 40°F. [a]

| Days storage | Weight loss in per cent | | | |
|---|---|---|---|---|
| | Washing treatment | | | |
| | Water | Triton X-100 | Olate flakes | Emulsifier "M" |
| 2 | 7.28 | 9.25 | 6.66 | 7.79 |
| 4 | 12.56 | 16.18 | 11.68 | 14.12 |
| 6 | 17.70 | 22.21 | 17.03 | 19.52 |

L.S.D. for detergent means within storage days -- 0.05 = 3.21

0.01 = 4.86

[a] Each value is the mean of three replications.

Analysis of variance indicated that weight losses after 4 and 6 days storage due to the use of the synthetic detergent, Triton X-100, were significantly greater, at the 0.05 level of probability, than the

losses due to the use of water or soap. Grouping of means showed that the use of the synthetic detergents, Triton X-100 and Emulsifier "M", resulted in weight losses significantly greater, at the 0.05 level, after 6 days storage than those which occurred in water-washed samples. These losses were also approaching significance at this level after 4 days storage. The synthetics also resulted in significantly greater losses than did the use of soap.

Residues

Two samples of 500 grams each were selected from each of the treatments, and the surface residues extracted by tumbling for 30 minutes in 1000 ml. of benzene in wide-mouth jars. After extraction and separation of the water layer, two 400 ml. aliquots of the benzene solution were placed in glass jars and stored at 30° to 40°F. until toxaphene and parathion analyses could be made.

Toxaphene residues remaining after the washing treatments are shown in Table 9. Values for check samples were at or near zero milligrams of toxaphene and are not included in this table; however, appropriate corrections have been made.

Washing in water alone removed a significant amount of the toxaphene that remained on the beans 24 hours after its application in the field. Emulsifier "M" removed a significant amount more residue than water washing. The amounts of residues remaining after washing with Triton X-100 and Olate flakes were significantly less, at the 0.01 level of probability, than those found after washing in water. Differences among blocks were highly significant, and were probably due to the heavy rainfall that preceded the harvest of the second block.

Table 9. - - Toxaphene residues on green beans following detergent washing - - Spring, 1953.

| Block | Toxaphene in mg. per 1000 grams of beans (ppm) [a] | | | | |
|---|---|---|---|---|---|
| | Washing treatment | | | | |
| | None | Water | Triton X-100 | Olate flakes | Emulsifier "M" |
| 1 | 20.53 | 20.53 | 12.32 | 10.34 | 15.76 |
| 2 | 21.72 | 14.84 | 7.99 | 7.29 | 8.92 |
| 3 | 23.99 | 15.99 | 9.78 | 10.13 | 13.45 |
| Treatment mean | 22.08 | 17.12 | 10.03 | 9.25 | 12.71 |

L.S.D. for treatment means - - 0.05 = 3.49

0.01 = 5.07

[a] Each value is the mean of duplicate determinations of two samples.

Parathion residues are shown in Table 10. Check samples carried through the analytical procedure developed little or no color, and were used to set the spectrophotometer at 100 per cent transmittance.

Water washing alone removed significant amounts of parathion from the beans sprayed 24 hours before washing. Detergents removed highly significant amounts of residue, and the differences between amounts removed by water and by detergents as a group approaches significance at the 0.05 level of probability. Highly significant differences among blocks are due almost entirely to the low values of Block 2 which was subjected to 1.4 inches of rain between the last spray application and harvesting.

Table 10. - - Parathion residues on green beans following detergent washing - - Spring, 1953.

| Block | Parathion in mg. per 1000 grams of beans (ppm) [a] | | | | |
|---|---|---|---|---|---|
| | Washing treatment | | | | |
| | None | Water | Triton X-100 | Olate flakes | Emulsifier "M" |
| 1 | 1.52 | 1.34 | 1.10 | 0.98 | 1.13 |
| 2 | 0.76 | 0.64 | 0.58 | 0.61 | 0.35 |
| 3 | 1.85 | 1.35 | 1.12 | 1.00 | 1.14 |
| Treatment mean | 1.38 | 1.11 | 0.93 | 0.86 | 0.87 |

L.S.D. for treatment means - - 0.05 = 0.27

0.01 = 0.39

[a] Each value is the mean of duplicate determinations of two samples.

Green Beans - - Fall, 1953.

The same experiment was repeated on a fall crop of beans of the same variety. Essentially the same cultural procedure was followed except that the beans received four applications of spray, the fourth being applied 12 hours prior to harvest. This reduction in time between spraying and harvesting was made in order to obtain greater residues of parathion. Due to the occurrence of soil-borne diseases, it was again necessary to combine adjacent blocks to obtain adequate harvests. The only appreciable rainfall during the season was 0.19 inch that fell immediately prior to the time the final spray was applied to the third block. Spray irrigations were applied when necessary and were timed so that several days elapsed between any spraying and irrigation. Mean temperatures were somewhat lower than they were during the spring. The minimums were from 38° to 58°F. with an average of 54°, and the maximums ranged from 66° to 88° and averaged 78°F. The mean temperature was 64°F. Only one block was harvested each day, and harvesting followed an application of spray to that block by 12 hours.

The washing treatments and procedures were the same as before. However, suitable storage facilities were not available, and no samples were held for quality evaluation.

Residue extractions and analyses were the same. In general, quantities of surface residues of toxaphene (Table 11) were greater than on the spring crop, except for Block 3, which was about the same. It is possible that a heavy covering of sand which resulted from the rain the same day the beans of this block received their last application of spray prevented adequate coverage by the spray. Water did not remove signifi-

Table 11. - - Toxaphene residues on green beans following detergent washing - - Fall, 1953.

| Block | Toxaphene in mg. per 1000 grams of beans (ppm) [a] | | | | |
|---|---|---|---|---|---|
| | Washing treatment | | | | |
| | None | Water | Triton X-100 | Olate flakes | Emulsifier "M" |
| 1 | 56.27 | 45.79 | 22.53 | 18.28 | 24.04 |
| 2 | 42.36 | 35.09 | 18.54 | 19.20 | 21.45 |
| 3 | 21.58 | 17.22 | 7.94 | 8.25 | 9.26 |
| Treatment mean | 40.07 | 32.70 | 16.34 | 15.24 | 18.25 |

L.S.D. for treatment means - - 0.05 = 10.05

0.01 = 14.62

[a] Each value is the mean of duplicate determinations of two samples.

cant amounts of residue. The amounts of residue remaining after detergent washings were significantly less, at the 0.01 level, than after water washing. Emulsifier "M" was an exception, but differences approached significance at this level. Block mean differences were highly significant, and probably account for the failure of mean differences between unwashed and water-washed samples being significant.

Highly significant amounts of parathion (Table 12) were removed by washing beans in water. The greater amounts of parathion residue removed by detergents as compared with water were not significant at the customary levels of probability, but they did approach significance at the 0.10 level. Differences between block means were highly significant

Table 12. - - Parathion residues on green beans following detergent washing - - Fall, 1953.

| Block | Parathion in mg. per 1000 grams of beans (ppm) [a] | | | | |
|---|---|---|---|---|---|
| | Washing treatment | | | | |
| | None | Water | Triton X-100 | Olate flakes | Emulsifier "M" |
| 1 | 5.17 | 2.94 | 2.62 | 2.42 | 2.57 |
| 2 | 2.25 | 1.47 | 0.87 | 1.08 | 1.10 |
| 3 | 2.70 | 1.12 | 0.78 | 0.75 | 0.83 |
| Treatment mean | 3.37 | 1.84 | 1.42 | 1.42 | 1.50 |

L.S.D. for treatment means - - 0.10 = 0.49

0.05 = 0.61

0.01 = 0.89

[a] Each value is the mean of duplicate determinations of two samples.

due to the greater values of Block 1. The cause for this is not known, but may be associated in some way with size of plants and foliage density.

Tomatoes - - Spring, 1953.

Homestead variety tomatoes were transplanted from a cold-frame to the field in Gainesville on March 23, 1953. The field plot was laid out in six replicated blocks, each 35 feet long. Plants were spaced 30 inches apart in eight rows 68 inches apart. The row most distant from power spraying in the direction of prevailing winds was used as a check row and received no insecticide sprays. The check row was protected on either side by a guard row, and a guard row separated the entire plot from other experiments. Vigor and general condition of the plants were good, although early fruit set was light, probably due to relatively cold weather at the time of the first bloom. Fungicidal sprays of wettable zineb were applied on April 6, 13, 20, and 27, and May 18. Wettable Spergon was applied on May 4 and May 11 in an attempt to control the heavy infestation of leaf mold, Cladisporium fulvum Cke., that had appeared. These fungicidal sprays were applied with a tractor-mounted power sprayer.

Toxaphene emulsion at a concentration of 2.5 pounds of active material per 100 gallons and parathion emulsion at a concentration of 0.5 pound of active material per 100 gallons were applied at a rate of 100 gallons per acre. Triton B-1956 at a concentration of 4 ounces per 100 gallons was used as a spreader-sticker. Insecticides were applied with a 3-gallon, compressed-air, hand sprayer. Insecticide spraying began on April 28, with one block per day being sprayed until the sixth block was sprayed on May 4. This sequence was repeated again beginning on May 12, 19, and 26. The first block was harvested on May 27, 24 hours after it had received a fourth application of insecticides, and the

sequence continued for the remaining blocks. This procedure was followed in order that washing treatments and residue extraction could be completed the same day the fruits were harvested. Block 3 received 1 inch of overhead irrigation between the last application of spray and picking. No other precipitation that might affect results occurred. Air temperatures during the time spray was on the plants were from 58° to 100°F., and from the time of the last spraying to harvesting were from 62° to 96°, averaging 81°F.

After the first harvest was completed, a second spray series was begun in which toxaphene was to be applied 3 days before harvest, and parathion 2 days prior to picking. General rains began just at this time and interfered with the timing of the sprays. Two blocks (4 and 5) were not harvested at all because of 1.97 inches of rain, and the timing of the toxaphene sprays of the remaining blocks was ruined because of other precipitation. Block 3 received 0.16 inch of rain 24 hours after receiving an application of parathion, and 0.05 inch fell on Block 6, 8 hours after it had been sprayed with parathion. Air temperatures from the time of the last spraying until harvesting of this second series were from 68° to 88° and averaged 76°F.

Tomatoes were harvested mature-green, and the fruits from each row were kept separate as they were picked. At the laboratory, the same number of fruit was randomly selected from each row harvest, thoroughly mixed, and divided into five lots. The same number of fruit was selected from the check-row harvest to constitute the sixth lot. The number of fruit selected from each row remained the same for all blocks of any harvest.

The tomatoes were placed in wire-mesh baskets and washed following the same procedure described above for beans and celery. Washing treatments, assigned to lots at random, and made in a random order, were: (A) unsprayed tomatoes washed in water, (B) sprayed tomatoes not washed, (C) sprayed tomatoes washed in water, (D) sprayed tomatoes washed in a 0.1 per cent solution of Triton X-100, (E) sprayed tomatoes washed in a 1.0 per cent solution of Olate flakes, and (G) sprayed tomatoes washed in a 0.1 per cent solution of Emulsifier "M". All solutions were calculated as per cent by weight, and temperatures were kept at about 72°F. A 10-minute washing period was followed by a thorough rinsing, and 2 hours of draining preceded extraction of the surface residues.

Quality

Ten tomatoes of each of the sprayed and washed treatments were weighed, placed in paper bags and stored for 6 days at 70°F. They were weighed and examined at 2-day intervals for possible effects of the detergents on their quality. Other than weight losses (Table 13), there were no apparent effects of detergents on color, odor, flavor, general appearance, or occurrence of injury. Some soil-rot lesions developed, but were apparently unaffected by washing treatments. Tomatoes of Block 6 of the second harvest that had been washed in water and those that had been washed in Triton X-100 were very badly decayed after 4 days storage, and this probably accounts for the excessive weight losses observed. After the general rains began, tomatoes of the second harvest developed very small cracks concentrically arranged around the stem end. These cracks had darkened considerably by the time the fruit were picked, and may have had an effect on the weight losses. The effects of detergents

Table 13. -- The effect of detergent washing on the weight loss of mature-green tomatoes during storage at 70°F.-- Spring, 1953

| Days storage | Weight loss in per cent | | | |
|---|---|---|---|---|
| | Washing treatment | | | |
| | Water | Triton X-100 | Olate flakes | Emulsifier "M" |
| | Harvest I [a] | | | |
| 2 | 1.15 | 1.28 | 1.11 | 1.16 |
| 4 | 2.10 | 2.20 | 1.98 | 2.17 |
| 6 | 3.18 | 3.09 | 2.86 | 3.27 |
| | Harvest II [bc] | | | |
| 2 | 1.43 | 1.44 | 1.24 | 1.26 |
| 4 | 2.62 | 3.60 | 2.20 | 2.23 |
| 6 | 3.94 | 5.42 | 3.27 | 3.28 |

Treatment differences are non-significant

[a] Each value is the mean of six replications.

[b] Each value is the mean of four replications.

[c] Not analyzed for statistical significance because of the high incidence of decayed fruit after 4 days storage.

on weight losses were not significant. Block 2, which was harvested when the general condition of the field was dry, showed less overall weight loss than other blocks.

Residues

Two samples of 10 fruit each were selected from each treatment, and the surface residue extracted by tumbling for 30 minutes in 1000 ml. of benzene in glass jars. Aliquots of 400 ml. of extractant were stored in glass jars at 30° to 40°F. for subsequent analyses of toxaphene and parathion.

The amounts of toxaphene residue remaining are shown in Table 14. Check samples had little or no residue and are not shown in the table; however, appropriate corrections were made wherever indicated.

Table 14. -- Toxaphene residues on mature-green tomatoes following detergent washing -- Spring, 1953.

| Block | Toxaphene in mg. per 1000 grams of tomatoes (ppm) [a] | | | | |
|---|---|---|---|---|---|
| | Washing treatment | | | | |
| | None | Water | Triton X-100 | Olate flakes | Emulsifier "M" |
| 1 | 3.90 | 4.50 | 3.87 | 3.95 | 2.23 |
| 2 | 4.37 | 3.54 | 3.18 | 3.77 | 2.99 |
| 3 | 4.56 | 3.56 | 3.55 | 3.60 | 3.53 [b] |
| 4 | 4.71 | 4.13 | 4.36 | 4.24 [b] | 3.80 |
| 5 | 4.28 | 3.53 | 3.11 | 4.64 | 4.77 |
| Treatment mean | 4.36 | 3.85 | 3.61 | 4.04 | 3.46 |

Treatment mean differences are non-significant

[a] Each value is the mean of duplicate determinations of two samples.

[b] Values obtained by application of missing plot formulae.

A slight reduction in the amount of residue found after detergent washing was noted. Differences, however, were not significant.

Although detergent effects on parathion residues (Table 15) were not significant, the block mean differences of Harvest II were significant at the 0.01 level of probability. This was due primarily to the low amount of residue found on fruit of Block 3, which had received 0.16 inch of rain 24 hours after the parathion was applied.

Table 15. -- Parathion residues on mature-green tomatoes following detergent washing -- Spring, 1953.

| Block | Parathion in mg. per 1000 grams of tomatoes (ppm) [a] | | | | |
|---|---|---|---|---|---|
| | Washing treatment | | | | |
| | None | Water | Triton X-100 | Olate flakes | Emulsifier "M" |
| | | | Harvest I | | |
| 1 | 0.26 | 0.15 | 0.26 | 0.27 | 0.37 |
| 2 | 0.19 | 0.32 | 0.26 | 0.21 | 0.22 |
| 3 | 0.29 | 0.28 | 0.26 | 0.34 | 0.28 |
| 4 | 0.39 | 0.32 | 0.17 | 0.27 | 0.15 |
| 5 | 0.39 | 0.21 | 0.32 | 0.29 | 0.31 |
| 6 | 0.48 | 0.25 | 0.28 | 0.34 | 0.31 |
| Treatment mean | 0.33 | 0.26 | 0.26 | 0.29 | 0.28 |
| | | | Harvest II | | |
| 1 | 0.28 | 0.30 | 0.24 | 0.19 | 0.10 |
| 2 | 0.29 | 0.26 | 0.30 | 0.28 | 0.26 |
| 3 | 0.14 | 0.12 | 0.15 | 0.14 | 0.13 |
| 6 | 0.40 | 0.30 | 0.28 | 0.37 | 0.34 |
| Treatment mean | 0.28 | 0.25 | 0.24 | 0.25 | 0.21 |
| Treatment mean differences are non-significant | | | | | |

[a] Each value is the mean of duplicate determinations of two samples.

Mustard Greens- - Fall, 1953.

Southern Giant Curled variety of mustard greens was planted in Gainesville on September 29, 1953, in eight rows. These rows were to be thinned later to form six replicated blocks, each 30 feet long. However, an early infestation of cabbage webworms, Hellula undalis Fabricius, destroyed such an area of the field that only three blocks could be formed. One row was separated from the others by guard rows, received no spray, and was designated as a check row. Normal cultural practices were followed, and except for the early attack by webworms, vigor of the plants was excellent, the yields good. There was some insect damage to the check, but it was of a minor nature. One-half inch of overhead irrigation was applied on November 3, 9, and 20. Rainfall of 2.2 inches was distributed over 7 days during the month of November, generally several days after any application of sprays. Minimum air temperatures during the time spray was on the plants were from 34° to 66° and averaged 52°F.; maximums were from 50° to 86° and averaged 72°F. The mean temperature was 60°F.

Toxaphene emulsion at a rate of 2.5 pounds active material per 100 gallons per acre and parathion at a rate of 0.5 pound of active material per 100 gallons per acre were applied at weekly intervals beginning October 26. Triton B-1956 at a concentration of 4 ounces per 100 gallons was used as a spreader-sticker. The last application before harvesting was split so that toxaphene could be applied 2 days before harvesting and parathion 12 hours prior to harvesting. This procedure was followed in order to reduce the amount of toxaphene residue and yet maintain a high parathion residue. The last application of toxaphene

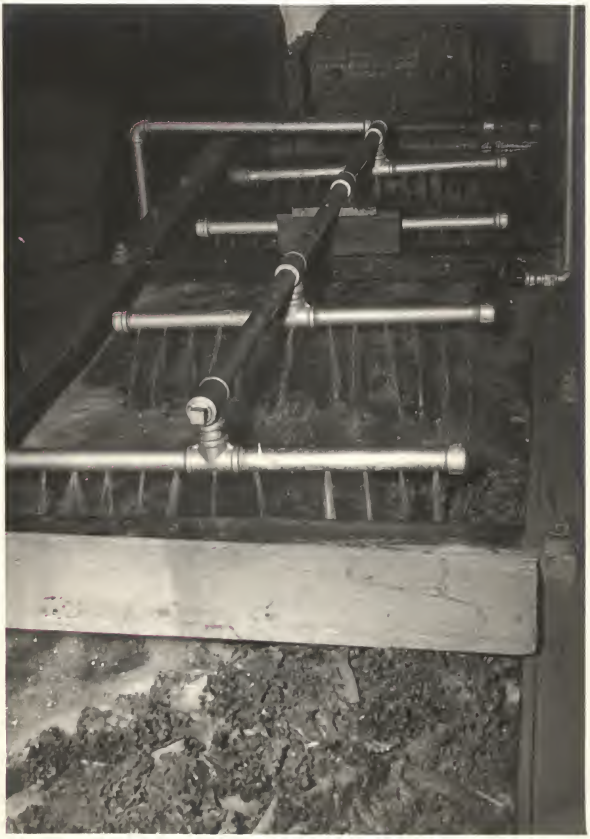
was applied to Block 1 on November 25, and the last parathion application was on November 26. This block was then harvested 12 hours later. The same procedure was followed in spraying and harvesting the other blocks with only one block being harvested on any one day.

As the mustard was cut, the greens from each row were kept separated, and at the laboratory the same quantity, by weight, from each row harvest was thoroughly mixed and divided into five lots of 6 pounds each. The same amount from the check row constituted the sixth lot. The six lots were designated and treated as follows: (A) unsprayed greens washed in water, (B) sprayed greens not washed, (C) sprayed greens washed in water, (D) sprayed greens washed in a 0.1 per cent solution of Triton X-100, (E) sprayed greens washed in a 1.0 per cent solution of Olate flakes, and (G) sprayed greens washed in a 0.1 per cent solution of Emulsifier "M". All solutions were calculated as per cent by weight, and temperatures were kept at 72°F. Designation of lots and treatment order was completely random.

The wash tank and spray-washer used for other crops were not suitable for washing the mustard because of its bulk. Therefore, stationary laundry tubs were used. The greens were soaked for 10 minutes in the washing solution, the greens being forced to the bottom of the tank every 2 minutes. After 10 minutes in the washing solution, the greens were rinsed by immersing them in three 10-gallon portions of water. They were then placed in wire baskets and allowed to drain for 2 hours.

Suitable storage facilities were not available for holding tests, but general observations did not indicate any adverse effects of the detergents on the quality of the mustard. All of the washed samples were

Fig. 8 - - Commercial Washing of Leafy Greens



well cleaned, and there were some indications of slight water-soaking in all treatments, but this feature had largely disappeared by the time residues were extracted. The mustard that had been washed in the synthetic detergent solutions was very crisp and turgid and could readily be distinguished from the other treatments.

Residues

The mustard greens, after draining, were chopped into 2- to 3-inch pieces, and duplicate 1000-gram samples taken for extraction of the surface residues. These were extracted by rotating for 30 minutes at 32 rpm. in 1000 ml. of benzene in wide-mouth gallon jars. Aliquots of the benzene solutions were placed in glass jars for storage until appropriate analyses were made.

Even though toxaphene residues (Table 16) were extremely high on unwashed mustard, the water treatment alone removed highly significant quantities. All the detergents removed further highly significant amounts and Triton X-100 and Olate flakes were significantly more effective than Emulsifier "M" at the 0.01 level of probability.

Parathion residues (Table 17) were also quite high. The water treatment, however, removed highly significant amounts, and all detergents removed highly significant quantities more than the water treatment alone. Differences among detergents were not significant.

Table 16. - - Toxaphene residues on mustard greens following detergent washing - - Fall, 1953.

| Block | Toxaphene in mg. per 1000 grams of mustard (ppm) [a] | | | | |
|---|---|---|---|---|---|
| | Washing treatment | | | | |
| | None | Water | Triton X-100 | Olate flakes | Emulsifier "M" |
| 1 | 193.80 | 109.82 | 19.38 | 28.42 | 58.14 |
| 2 | 206.72 | 88.17 | 19.38 | 14.53 | 49.10 |
| 3 | 300.39 | 102.07 | 32.30 | 19.38 | 52.16 |
| Treatment mean | 233.64 | 100.02 | 23.69 | 20.77 | 53.13 |

L.S.D. for treatment means - - 0.05 = 15.62

0.01 = 22.71

[a] Each value is the mean of duplicate determinations of two samples.

Table 17. - - Parathion residues on mustard greens following detergent washing - - Fall, 1953.

| Block | Parathion in mg. per 1000 grams of mustard (ppm) [a] | | | | |
|---|---|---|---|---|---|
| | Washing treatment | | | | |
| | None | Water | Triton X-100 | Olate flakes | Emulsifier "M" |
| 1 | 26.40 | 10.08 | 4.70 | 3.20 | 5.40 |
| 2 | 23.50 | 8.70 | 3.81 | 3.15 | 5.07 |
| 3 | 28.00 | 8.20 | 4.63 | 3.80 | 4.45 |
| Treatment mean | 25.96 | 8.99 | 4.38 | 3.38 | 4.97 |

L.S.D. for treatment means 0.05 = 2.10

0.01 = 3.05

[a] Each value is the mean of duplicate determinations of two samples.

## DISCUSSION

The use of filter papers as a surface to hold insecticides for laboratory treatments for the purpose of screening large numbers of washing materials appeared to be feasible. This laboratory method should not be used for other than screening tests for the elimination of materials ineffective under these conditions unless subsequent modifications are made. The method did not give any information that would indicate the absolute amount of residue that would be removed from any vegetable, nor was such information expected. It did show that certain detergents were more effective, in themselves, than others under similar conditions; moreover, these relationships were fairly well corroborated by tests with treated vegetables. The absolute amount of any insecticide held by filter paper in such a way as to be difficult to remove would certainly be different from the amount securely held by tomatoes, beans, or any other fruit or vegetable with a different adsorbing surface.

The number of detergents used on vegetables and the number of replications were not adequate to permit the use of statistical tests to correlate the results of paper washing with results of vegetable tests. However, general observations would indicate that, with the exception of tomatoes, the amount of residues removed, expressed as a percentage of the original concentration, differed somewhat between vegetables and between vegetables and papers, but differences between detergents remained essentially the same. The order of effectiveness of Triton X-100 and Olate flakes was apparently reversed when applied to vegetables, but this may have been due to concentration differences, equipment used,

plant effects, or sampling variation. Differences between the two materials were non-significant in every case. The calculated percentages of residues removed are given in Tables 18 and 19, and shown in Figs. 9 and 10.

Detergent washes were effective in removing surface residues of toxaphene from fresh vegetables. They were able to remove significantly more toxaphene than water used alone except from tomatoes. Initial residues on tomatoes however were less than 5 ppm. In no case did detergent treatments reduce the amount of toxaphene to less than 5 ppm when the initial amount was above that level. However, some treatments did approach that final amount of residue, which is an unofficial estimate of the maximum allowable tolerance for toxaphene which may be established sometime in the future by the Food and Drug Administration. It is quite likely that these materials under different conditions, such as longer washing time, brushing, higher washing temperatures, or a greater volume of water, would reduce residues to less than that amount. In any case, however, the general level of residues remaining on the crops to be ultimately consumed was substantially reduced.

Detergents were significantly more effective than water washing for removing surface residues of parathion only in the case of mustard greens. However, the differences on beans approached significance at the 0.05 level, and with increased replications may have surpassed the customary levels of probability. Surface residues of parathion on tomatoes were less than 0.5 ppm, and were no greater 1 day after spray application than they were when the spray was applied 2 days before harvesting. In as much as Gunther et al (18) have reported the absorption

Table 18. - - The effect of detergent washing on the removal of toxaphene residues from the surfaces of treated materials.

| Treated material | Residue in per cent removed by | | | | | Per cent more removed than by water | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Water | Triton X-100 | Olate flakes | Emulsifier "M" | Tergitol NPX | Triton X-100 | Olate flakes | Emulsifier "M" | Tergitol NPX |
| Green beans I | 22.46 | 54.58 | 58.11 | 42.44 | - - - | 32.12 | 35.65 | 19.98 | - - - |
| Green beans II | 18.39 | 59.22 | 61.97 | 54.46 | - - - | 40.83 | 43.58 | 36.07 | - - - |
| Tomatoes | 11.70 | 17.20 | 7.34 | 20.65 | - - - | 5.50 | -4.36 | 8.95 | - - - |
| Celery [a] | 57.30 | 71.89 | 72.83 | - - - | 68.68 | 14.59 | 15.53 | - - - | 11.38 |
| Mustard greens | 57.19 | 89.86 | 91.11 | 72.26 | - - - | 32.67 | 33.92 | 20.07 | - - - |
| Average | | | | | | 25.14 | 24.86 | 21.26 | 11.38 |
| Papers | < 4.07 | 28.75 | 27.11 | 21.52 | 22.50 | 24.68 | 23.04 | 17.45 | 18.43 |

[a] Only the results of washing celery of the first harvest are included in these values.

Table 19. -- The effect of detergent washing on the removal of parathion residues from the surfaces of treated materials.

| Treated material | Residue in per cent removed by | | | | Per cent more removed than by water | | |
|---|---|---|---|---|---|---|---|
| | Water | Triton X-100 | Olate flakes | Emulsifier "M" | Triton X-100 | Olate flakes | Emulsifier "M" |
| Green beans I | 19.57 | 32.61 | 37.68 | 36.96 | 13.04 | 18.11 | 17.39 |
| Green beans II | 45.40 | 57.86 | 57.86 | 55.49 | 12.46 | 12.46 | 10.09 |
| Tomatoes I | 21.21 | 21.21 | 12.12 | 15.15 | 0.00 | -9.09 | -6.06 |
| Tomatoes II | 10.71 | 10.71 | 10.71 | 25.00 | 0.00 | 0.00 | 14.29 |
| Mustard greens | 65.37 | 83.13 | 86.98 | 80.86 | 17.76 | 21.61 | 15.49 |
| Average | | | | | 8.65 | 10.77 | 8.93 |
| Papers | < 3.33 | 22.70 | 21.42 | 25.55 | 19.37 | 18.09 | 22.22 |

Fig. 9 - - The effect of detergent washing on the removal of surface residues of toxephene from treated materials.

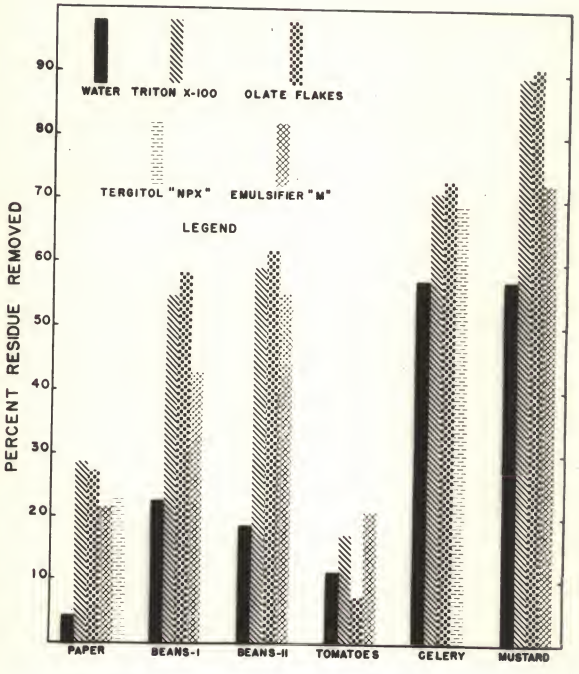
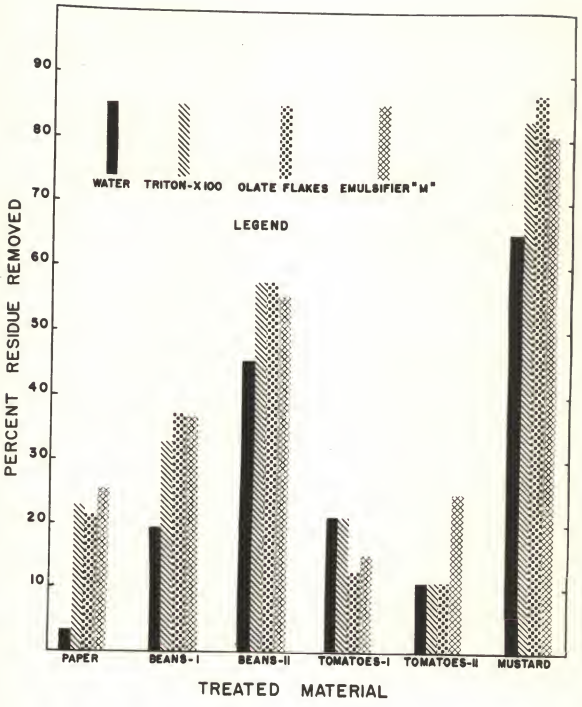

WATER
TRITON X-100
OLATE FLAKES
TERGITOL "NPX"
EMULSIFIER "M"
LEGEND
PERCENT RESIDUE REMOVED
90
80
70
60
50
40
30
20
10
PAPER
BEANS-I
BEANS-II
TOMATOES
CELERY
MUSTARD
TREATED MATERIAL

Fig. 10 - - The effect of detergent washing on the removal of surface residues of parathion from treated materials.


90
80
70
60
50
40
30
20
10
PERCENT RESIDUE REMOVED
WATER
TRITON-X 100
OLATE FLAKES
EMULSIFIER "M"
LEGEND
PAPER
BEANS-I
BEANS-II
TOMATOES-I
TOMATOES-II
MUSTARD
TREATED MATERIAL

of parathion by citrus fruits due to its solubility in plant oils and waxes, the possibility of sub-surface residues occurring on such crops as tomatoes and beans should be further investigated. However, most of the variation in results on tomatoes here is believed to be due to sampling variation caused by the nature of the fruit, plant variations, and handling techniques.

Although varying amounts of initial residues were limited in these experiments, results seem to indicate, as might be expected, that the greater the residue per unit of surface, the greater the per cent removal. For example, the results with parathion residues on green beans: water was able to remove only 20 per cent of an initial quantity of 1.38 ppm, but was able to remove 45 per cent of an original amount of 3.37 ppm. If this was a true difference and not sampling variation, it would indicate a need for the development of spray equipment that could uniformly deposit an effective but not excessive covering of spray materials on the plant surfaces. This would not only result in a reduction of possible residue hazards, but would seem to offer a means of reducing insecticide costs.

It should be emphasized that the spray programs followed in this work were in no way considered as economically or practically desirable, but served only to provide maximum quantities of residues. Nevertheless, results indicated that the possibility exists, particularly for the producers of crops for processing, for the application of insecticides up to a few days prior to harvest with the assurance that residues could be markedly reduced. Much additional work is necessary, however, for this possibility to become a reality.

Weight losses of vegetables during storage after treatment with synthetic detergents were generally greater than for vegetables treated with soap or water. Although no examinations were made, this was possibly due to the effect of the detergents on the surface oils and waxes of the vegetables and a resulting increase in transpiration. Green beans were injured by very low concentrations of detergents, and other crops may be damaged by higher concentrations. Since the toxicity of many detergents has not been established, the indiscriminate use of detergents for the washing of fresh vegetables is not suggested.

SUMMARY

Laboratory equipment and methods were devised for screening a large number of washing materials to determine their effectiveness in removing residues of toxaphene and parathion from treated surfaces. The procedure used consisted of subjecting treated filter papers to a standard washing and rinsing, and measuring the amount of residues on the papers before and after treatment.

Of fifteen specific compounds or materials representative of a type of compound tested by these methods, only four were considered effective enough to be used in tests on vegetable crops treated with the same insecticides.

Water washing alone in small-scale equipment in the laboratory removed more than 50 per cent of a toxaphene residue of 75 to 100 ppm from celery. Detergents were able to remove approximately 70 per cent of the toxaphene residue. One per cent solutions of the synthetic detergents used resulted in excessive losses of weight when the celery was held in cold storage after washing treatments.

Toxaphene residues of 20 to 40 ppm on green beans were reduced 20 per cent by water washing and 40 to 60 per cent by detergent washes. Residues of 1 to 2 ppm of parathion were reduced 20 per cent by water and 30 to 35 per cent by detergents, and parathion residues of 3 to 5 ppm were reduced 45 per cent by water, while 55 to 60 per cent reductions resulted from the use of detergents. Weight losses during subsequent cold storage were great, but differences due to washing materials used were not significant.

Tomatoes bearing a toxaphene residue of 4 ppm were not significantly affected by detergent treatments. There was only a slight reduction in the amount of residues by any treatment. Parathion residues on tomatoes 1 day after spraying were less than 0.5 ppm, and were not significantly reduced by washing treatments.

Toxaphene residues on mustard greens 2 days after spraying were in excess of 200 ppm, but washing in water removed 57 per cent of that residue, and washing in detergents removed 70 to 90 per cent of the residue. Parathion residues 12 hours after spraying were 25 ppm, but were reduced 65 per cent by water and 80 to 85 per cent by detergents.

In general Triton X-100 and Olate flakes were the most effective washing agents, and differences between the two were always very slight. In most cases, however, weight losses of the produce during subsequent storage periods were greater when Triton was used. In the case of green beans, Triton also resulted in some injury to the vegetable. The effective use of this material, therefore, is conditioned by the subsequent treatment and handling to be given the treated materials.

Detergent washes, in general, were more effective in removing residues of toxaphene and parathion from the surface of sprayed vegetables than water alone. Many problems of application remain, however, before recommendations for the use of any material or method can be made.

BIBLIOGRAPHY

1. Adams, R. E. Leaf punch for sampling foliage for analysis of spray deposits. Phytopath. 41:568-569. 1951.

2. Averell, P. R., and M. V. Norris. Estimation of small amounts of O,O-diethyl,O-p-nitrophenyl thiophosphate. Anal. Chem. 20:753-758. 1948.

3. Baier, W. E., and C. W. Wilson. Proposed basis for tolerances of insecticides. Food Tech. 5:38-40. 1951.

4. Bowen, C. V., and F. I. Edwards. Polarographic determination of parathion. Anal. Chem. 22:706-708. 1950.

5. Britten, W. A. Report on insecticides in canned foods. Effect of processing on spray residue. J. Assn. Off. Agr. Chem. 33:599-607. 1950.

6. Carter, R. H., R. H. Nelson, and W. A. Gersdoff. Organic chlorine determinations as a measure of insecticide residues in agricultural products. Advances in Chemistry Series 1: 271-273. 1950.

7. Cassaday, J. T., J. H. Fletcher, J. C. Hamilton, I. Hechenbleikner, E. I. Hoegberg, B. J. Sertl, and J. T. Thurston. Synthesis and development of parathion and related compounds. Advances in Chemistry Series 1:143-149. 1950.

8. Cupples, H. L. Relationship between wetting power of a spray and its retention on a fruit surface. J. Agr. Res. 63:681-686. 1941.

9. Decker, G. C., C. J. Weinman, and J. M. Bann. Preliminary report on the rate of insecticide residue loss from treated plants. J. Econ. Ent. 43:919-927. 1950.

10. Fahey, J. E. Estimation of undecomposed DDT spray deposits on apples from total organic chlorine. J. Assn. Off. Agr. Chem. 28:152-158. 1945.

11. Gage, J. C. The analysis of p-nitrophenyl diethyl thiophosphate. Analyst 75:189-191. 1950.

12. Gaines, J. C., and W. J. Mistric, Jr. Effect of rainfall and other factors on the toxicity of certain insecticides. J. Econ. Ent. 44:581-585. 1951.

13. Ginsberg, J. M., R. S. Filmer, and J. P., Reed. Longevity of parathion, DDT, and DDD residues on field and vegetable crops. J. Econ. Ent. 43:90-94. 1950.

14. _____, and A. R. Patterson. Recovery of parathion, DDT, and DDD from treated crops. J. Econ. Ent. 42:602-611. 1949.

15. Gould, W. A. Flavor evaluation for canned fruits and vegetables treated with newer organic insecticides. Food Tech. 5: 129-133. 1951.

16. Griffiths, J. T., J. W. Williams, W. L. Thompson, and C. R. Stearns, Jr. Toxicology of parathion and other phosphatic insecticides and precautions for their use on citrus. Florida Agr. Exp. Sta. Bul. 479. 1951.

17. Gunther, F. A., and R. C. Blinn. Mass production techniques for estimation of parathion residues. Advances in Chemistry Series 1:72-87. 1950.

18. Gunther, F. A., M. M. Barnes, and G. E. Carman. Removal of DDT and parathion residues from apples, pears, lemons, and oranges. Advances in Chemistry Series 1:137-142. 1950.

19. Hall, S. A. Organic phosphorus insecticides. Advances in Chemistry Series 1:150-159. 1950.

20. Haller, H. L. Wartime development of insecticides. Ind. and Eng. Chem. 39:567-573. 1947.

21. _____. Chemical aspects of some of the newer insecticides. Bul. Assn. Food and Drug Off. 13:374-381. 1949.

22. Handbook on Pesticides and their uses in Florida agriculture. Florida Agr. Exp. Sta., Florida Agr. Ext. Ser., Florida Industry Organics Committee, U. S. Fish and Wildlife Ser. 131 pp. 1951.

23. Hartsell, A., and E. E. Storrs. Bioassay of insecticide spray residue in processed food. Contr. Boyce Thompson Inst. 16:47-53. 1950.

24. Hazleton, L. W., and E. G. Holland. Pharmacology and toxicology of parathion. Advances in Chemistry Series 1:31-38. 1950.

25. Hoskins, W. M. Deposit and residue of recent insecticides resulting from various control practices. J. Econ. Ent. 42:966-973. 1949.

26. Kolthoff, I. M., and P. K. Kuroda. Argentometric amperometric titration of traces of chlorides. Anal. Chem. 23:1306-1309. 1951.

27. Laakso, J. W., and L. H. Johnson. Toxaphene residues on alfalfa. Montana Agr. Exp. Sta. Bul. 461:5-15. 1949.

28. Lackey, R. W. Observations on the percutaneous absorption of toxaphene in the rabbit and the dog - - II. J. Ind. Hyg. Toxicol. 31:155-157. 1949.

29. Laitenin, H. A., W. P. Jennings, and T. D. Parks. Amperometric titration of chloride, bromide, and iodide using the rotating platinum electrode. Ind. and Eng. Chem., Anal. Ed. 18:355-358. 1946.

30. Landis, O., and H. J. Wichman. Decomposition of organic chlorides with sodium in alcohol. Ind. and Eng. Chem., Anal. Ed. 2:394. 1930.

31. Laudani, H., and F. O. Marake. Toxicity to fabric insects and resistance to washing and dry cleaning of six chlorinated hydrocarbon insecticides. J. Econ. Ent. 42:434-436. 1949.

32. Lauger, P., H. Martin, and P. Muller. Uber Konstitution und toxische Wirkung von naturlichen und neuen synthetischen insektentotenden Stoffen. Helv. Chem. Acta. 27:892-928. 1944.

33. Lehman, A. J. The toxicology of the newer agricultural chemicals. Bul. Assn. Food and Drug Off. 12:82-89. 1948.

34. _____. The major toxic actions of insecticides. Bul. New York Acad. Med. 25:382-387. 1949.

35. _____. List of materials used on agricultural products. Food Ind. 22:1266-1267. 1950.

36. List of Pesticidal Chemicals Used on Fresh Fruits and Vegetables. Chem. and Eng. News. 27:3876-3877. 1949.

37. Manalo, G. D., R. Hutson, E. J. Miller, and E. J. Benne. Removal of DDT spray residues from apples. Michigan Agr. Exp. Sta. Quar. Bul. 29:15-22. 1946.

38. Martin and Shaw. British Intelligence Objectives Sub-committee, BIOS Final Rept. 1095, Item 22. 1946.

39. McBain, J. W., and P. H. Richards. Solubilization of insoluble organic liquids by detergents. Ind. and Eng. Chem. 38:642-646. 1946.

40. McCutcheon, J. W. Synthetic detergents - - up to date. J. W. McCutcheon, New York. 36 pp. 1949.

41. McCutcheon, J. W. Synthetic detergents - - up to date - - II - - 1952. J. W. McCutcheon, New York. 41 pp. 1952.

42. Metcalf, C. L., and W. P. Flint. Destructive and useful insects. Second Edition. McGraw-Hill Book Co., New York. 981 pp. 1939.

43. Nolan, K., and F. Wilcoxon. Method of bioassay for traces of parathion in plant material. Agr. Chem. 5:53-56. 1950.

44. Parathion Tolerance Data. Federal Security Agency Docket FDC-57. 124 pp. 1950.

45. Peairs, L. M. Insect pests of farm, garden, and orchard. John Wiley and Sons. New York. 549 pp. 1947.

46. Riker, A. J. The preparation of manuscripts for Phytopathology. Phytopath. 36:953-977. 1946.

47. Rowher, S. A. Toxaphene, a common name for the insecticidal chemical, chlorinated camphene. J. Econ. Ent. 42:225. 1949.

48. Ruth, W. A. Apple washing machinery. Illinois Hort. 31:4-5. 1942.

49. Schrader, A. L., and M. H. Haller. Comparison of wetting agents in apple washing. Proc. Amer. Soc. Hort. Sci. 36:243-246. 1938.

50. Scruggs, F. H., and E. F. Scarborough. Annual fruit and vegetable report. 1952-53. Florida State Marketing Bureau. Jacksonville, Florida. 132 pp. 1953.

51. Sloan, M. J., W. A. Rawlins, and L. B. Norton. Factors affecting the loss of DDT and parathion residues on lettuce. J. Econ. Ent. 44:701-709. 1951.

52. Spray Residues on Tomatoes - Acid Dip Method of Removal. Agr. Gaz. New South Wales 58:409-410. 1947.

53. Stasch, A. R. A study of toxaphene residues. Masters thesis (unpublished). Univ. of Florida Library. 67 pp. 1952.

54. Stansbury, H. E., and P. A. Dahm. Effect of alfalfa dehydration upon residues of aldrin, chlordan, DDT, parathion, and toxaphene. J. Econ. Ent. 44:45-51. 1951.

55. Taschenberg, E. F., and A. W. Avens. DDT residue studies on grapes, jam, and juice. New York State Agr. Exp. Sta. Tech. Bul. 286. 1949.

56. Toxaphene Manual - - Analytical Procedure. Hercules Powder Co., Wilmington, Delaware. 39 pp. 1951.

57. Toxicity of Insecticides, Fungicides, and Herbicides - - report of current research and research needs. U.S.D.A. Agr. Res. Adm. 68 pp. (mimeo.) 1951.

58. Umhofer, R. H. The determination of halogens in organic compounds. Ind. and Eng. Chem., Anal. Ed. 15:383-384. 1943.

59. Vinson, E. B., and F. S. Arant. Parathion, toxaphene, and DDT residues on peanut hay. J. Econ. Ent. 43:942-943. 1950.

60. Walker, K. C. DDT residue and its removal from apples and pears. Proc. Amer. Soc. Hort. Sci. 51:85-89. 1948.

61. _____. Problems relating to the removal of DDT from apples. J. Agr. Res. 78:383-387. 1949.

62. Wilson, C. W., R. Baier, D. Genung, and J. Mullowney. Estimation of O,O-diethyl,O-p-nitrophenyl thiophosphate. Anal. Chem. 23:1887-1889. 1951.

63. Wilson, J. W., and N. C. Hayslip. Insects attacking celery in Florida. Florida Agr. Exp. Sta. Bul. 486. 1951.

# APPENDIXES

## APPENDIXES

### APPENDIX A - - METHODS OF CHEMICAL ANALYSES

Analytical methods for the determination of toxaphene and parathion are relatively recent introductions; therefore, they are given below in detail. The basic procedures, from the indicated sources, were tested and modified to meet the conditions of this work. Certain difficulties that were encountered and some general observations on the use of these methods are presented as being of possible value to future work.

#### Toxaphene

In principle this method depends upon the determination of the amount of chlorine present, and calculation of the toxaphene content based on the minimum chlorine content of technical toxaphene. The insecticide, in a solution of isopropanol, is decomposed by metallic sodium, and the resulting chlorides determined by argentometric amperometric titration. Chlorine from other sources such as other insecticides and fertilizers will also be determined and may be erroneously calculated as toxaphene.

The basic procedure is taken largely from the Toxaphene Manual of the Hercules Powder Company (56).

Reagents

(1) Isopropanol, dry 99% isopropanol with anhydrous sodium sulfate.

(2) Metallic sodium, reagent grade.

(3) Nitric acid, 1:1 water mixture, by volume.

(4) Gelatin solution, 1% aqueous.

(5) Silver nitrate, 0.02 to 0.005 N accurately standardized.

(6) Acetone, A.C.S., reagent grade.

(7) Hydrogen peroxide, 30% (Merck Superoxol or equivalent).

Procedure

After the surface residue on the vegetables had been extracted with benzene, aliquots of the extract solution were taken and were evaporated to about 75 ml. volume using a steam bath and an air stream to facilitate evaporation. The solution was transferred to a 300 ml. Erlenmeyer flask with a ground-glass neck, and the evaporation continued to a volume of 5 to 10 ml., being careful not to take to dryness. To this solution was added 25 ml. of isopropanol and 2.5 grams of sodium cut into small (1/8") pieces. The entire mixture was then refluxed for 30 minutes using a hot-plate and water-jacketed condensers with ground-glass connections. The excess sodium was cautiously destroyed by adding 50 per cent aqueous isopropanol dropwise, and refluxing an additional 10 minutes. Occasionally more alcohol was required to destroy all of the sodium. To oxidize traces of sulfides that may have come from sulfur sprays or other sources, 10 ml. of 30 per cent hydrogen peroxide were added through the condenser, and the mixture refluxed for 30 minutes. The solution was allowed to cool, the condensers were washed down with 10 to 20 ml. of distilled water, and the solution transferred to a 250 ml. beaker. Using a steam bath and a stream of air, most of the alcohol layer was then evaporated. The solution was acidified with the 1:1 nitric acid to the phenolphthalein end point, and 10 ml. excess added. Then 10 ml. of gelatin solution and sufficient acetone to make a 50 per

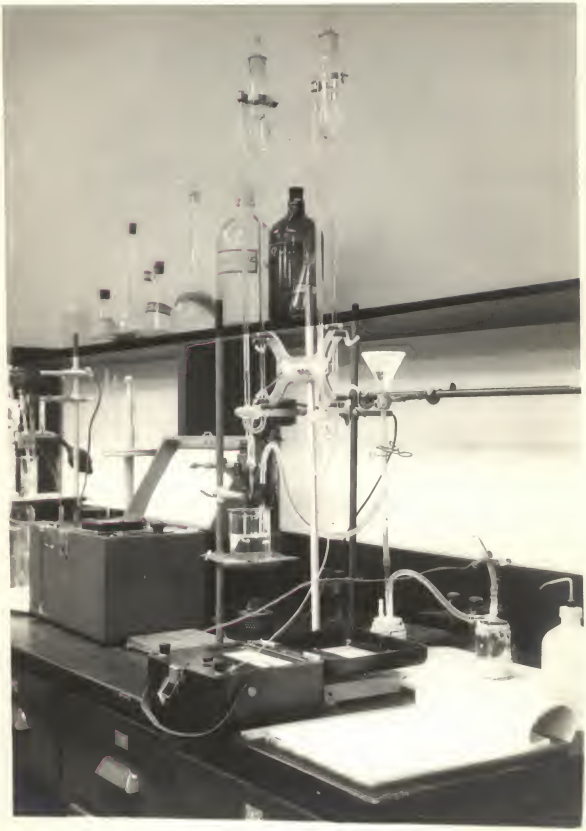
cent acetone solution were added. The chlorides were titrated with standardized silver nitrate using amperometric technique.

As applied to the titration of chlorides, amperometric titration is essentially the measurement of the current which passes through the solution between a rotating platinum microelectrode and a saturated-calomel reference electrode. This current is plotted as a function of the volume of standard silver nitrate added. The end point is found at the intersection of the two straight lines showing the current change before and after the equivalence point. To avoid contamination of the solution being titrated by diffusion of the KCl of the reference electrode, a $KNO_3$ pool was used as an intermediate step in the salt bridge. The apparatus used is shown in Fig. 11.

The beaker was placed in position for titration, and rotation of the platinum microelectrode at a constant speed by a suitable motor begun. After the system came to equilibrium (2 to 10 minutes), the first reading of the microammeter was taken, 0.25 ml. of standardized silver nitrate added from a microburet, and the ammeter reading recorded. The system was allowed to come to equilibrium before readings were made. As a standard procedure, readings were taken after the addition of 0.25, 0.50, 0.75, and 1.00 ml. of silver nitrate. Then more reagent was added until a large permanent deflection of the ammeter was observed. After the system reached equilibrium, the reading was taken, and small additional amounts of silver nitrate added until three or four readings beyond the end point were made.

After each titration, several milliliters of $KNO_3$ solution were permitted to flow out through the bridge terminal to aid in maintaining

Fig. 11 - - Amperometric Titration Assembly



the stability of the circuit by preventing the contamination of the $KNO_3$ solution by diffusion from the titration beaker.

Recovery of Added Toxaphene

Standard solutions containing from 0.20 to 50.00 mg. of toxaphene per 250 ml. of benzene were analyzed by the above procedures. The results, given in Table 20, showed an average recovery of 97.69 per cent if the concentrations below 2.00 mg. were ignored. This recovery did not differ greatly from other published results (53), and, since it was felt that accuracy and precision would improve with practice, this procedure was followed. The major variation from other reports was in the range of concentration below 2.00 mg. According to the manual (56) cited above, it is often desirable to add known amounts of chloride to bring the level of analysis to a more precise range, and this recommendation was followed whenever less than 3.00 mg. of toxaphene were anticipated.

Modifications in Procedures

During the analysis of samples extracted from celery it became apparent that sub-sampling variability was excessive and was causing somewhat erratic results. Dr. A. H. Gropp of the Department of Chemistry suggested that some steps in the procedure could be causing this, and several tests were made incorporating these suggestions as changes in the procedure.

Standard solutions of KCl containing from 0.10 to 50.00 mg. of chloride were analyzed by the original procedure except that a pH meter was used to adjust the solution to a pH value of 8.6 using 1:1 nitric acid. An additional 10 ml. of acid were then added. Gelatin solutions

Table 20. - - Recovery of added amounts of toxaphene

| Toxaphene in mg. | | Per cent recovery |
|---|---|---|
| Added | Found [a] | |
| 0.20 | 0.46 | 230.00 |
| 0.50 | 0.67 | 134.00 |
| 2.00 | 2.07 | 103.50 |
| 5.00 | 4.52 | 90.40 |
| 10.00 | 10.24 | 102.43 |
| 20.00 | 19.80 | 99.00 |
| 50.00 | 46.56 | 93.12 |
| Average per cent recovery - - - - - - - | | 97.69 [b] |

[a] Each value is the mean of three determinations except values of 2.00 mg. and below are means of six determinations.

[b] Does not include per cent recovery of amounts less than 2.00 mg.

were made in small quantities so that fresh solutions could be used each day. Results obtained by using this procedure are shown in Table 21.

The same procedure was followed in analyzing standard solutions of toxaphene, and resulted in an average recovery of 99.70 per cent. Amounts of toxaphene less than 3.00 mg. were not detected.

By avoiding the addition of phenolphthalein to the titration solution and using only freshly prepared gelatin solutions plus expertly constructed platinum microelectrodes, general performance of the method appeared to be better, although recoveries were not greatly different.

Table 21. - - Recovery of chlorides by amperometric titration methods.

| Chloride in mg. | | Per cent recovery |
|---|---|---|
| Added | Found [a] | |
| 0.10 | 0.00 | - - - |
| 0.30 | 0.00 | - - - |
| 0.50 | 0.30 | 60.00 |
| | 0.00 | - - - |
| 0.70 | 0.60 | 85.71 |
| 1.00 | 0.83 | 83.00 |
| 2.00 | 1.97 | 98.50 |
| 4.00 | 4.03 | 100.75 |
| 5.00 | 5.01 | 100.50 |
| 10.00 | 10.29 | 102.90 |
| 15.00 | 15.69 | 104.60 |
| 20.00 | 20.95 | 104.75 |
| 50.00 | 52.96 | 105.92 |
| Average per cent recovery - - - - - - | | 103.24 [b] |

[a] Each value is the mean of three determinations.

[b] Does not include per cent recovery of amounts less than 2.00 mg.

Amperometric techniques have been used successfully to accurately determine chlorides as dilute as $3 \times 10^{-5}$ N (56). However, under the conditions of these tests such dilutions were not accurately measured. Results of plotting the above data indicated the range of greatest

accuracy to be centered at about 5.00 mg. of chloride; therefore, subsequent aliquots were based on this desired amount. Five milligrams of chloride from standard solutions of KCl were added to any samples that were expected to contain less than this amount.

The evaporation of 250 to 500 ml. of benzene from each aliquot often required 6 to 8 hours time, much inconvenience, and resulted in a great loss of benzene. Several tests were made using standard solutions of toxaphene, and evaporating the samples by means of Friedrichs distillation condensers. Approximately 90 per cent of the solvent was recovered by evaporating to a volume of 25 to 50 ml. before transferring to a 300 ml. Erlenmeyer flask for evaporation to a volume of 5 to 10 ml. prior to sodium decomposition. These tests showed that there was no apparent loss of toxaphene by concentration in this apparatus, which is shown in Fig. 12, and this modification was adopted.

These modifications still did not account for all the variability encountered, and it was not until benzene that had been redistilled in the laboratory was checked that the major source of error was discovered. At some time in the extensive operation of the laboratory, redistilled benzene had inadvertently been stored in cans that had previously been used for waste benzene and other solvents. The use of this benzene at irregular times during sample extraction and in laboratory procedures was undoubtedly the major source of error in analyses of celery samples. Analysis indicated that some of this benzene contained more than 10 mg. of chloride per ml.

Fig. 12 - - Freidrichs condensers used to concentrate large aliquots of extract solutions.

Observations

These observations are entirely personal views that have developed during the course of the analysis of many hundreds of samples, and in most cases are not results of specific tests.

The sodium-isopropanol decomposition of the insecticide appeared to give good results. It was relatively rapid and simple and well adapted to routine work. Sodium shot approximately 8 to 9 mm. in diameter would be very convenient and is now on the market.

Unitized apparatus for amperometric titrations has been offered recently by supply houses, and would certainly be desirable for any future work.

A generally sluggish response of the amperometric titration assembly seemed to indicate that the pool of $KNO_3$ required replacement, or that the calomel electrode should be changed. A large deflection of the ammeter at the approximate equivalence point followed by a slow but steady return to about the original reading was an indication that an air bubble had entered the system or that the sintered glass plugs were closed by a precipitate. The most satisfactory remedy was to clean and remake the entire apparatus. A steady increase in the ammeter reading near the end point that rarely stopped before reaching the maximum scale reading generally indicated a crack in the glass wall of the platinum microelectrode. Platinum microelectrodes are now being offered for sale by supply houses. The response of the apparatus to the addition of reagent was normally such that over-titrating seldom occurred unless such a crack had appeared. Many times the original current registered on the ammeter was so high that a good titration was

impossible. Some of the equipment now offered for sale is designed to permit adjustment of resistance to bring the readings to such a position on the scale that accurate measurements can be made. The author was never able to do this with the expedients available. A possible cause for some of these high readings might have been copper salts from the copper tubing that was used as an air manifold for evaporation of some of the earlier samples. These high initial readings also seemed to indicate that the calomel electrode should be replaced.

The presence of parathion residues apparently had no effect on the analysis of toxaphene.

## Parathion

A specific method for the analysis of parathion was developed by Averell and Norris (2), and modified by Gunther and Blinn (17) for mass production techniques of spray residue analysis. This method is based on the reduction of the nitro group to the amine, diazotization, and coupling with N-(1-naphthyl)ethylenediamine which gives an intense magenta color that can be quantitatively measured with a spectrophotometer.

The procedure given below is taken from Gunther and Blinn (17) with some adaptations suggested by workers at the Florida Agricultural Experiment Stations.

### Procedure

To the aliquot from the extracting solvent were added approximately 10 grams, per 100 ml. of solvent, of a finely ground 2:1 mixture of Attapulgus clay and Hyflo Super-Cel. This quantity of decolorizing material was generally adequate for such strongly colored extracts as

those from leafy, green vegetables, and was reduced for less intensely colored extracts. The solutions were shaken in a mechanical soil shaker for 15 minutes, and then filtered through Sharkskin paper. Suitable aliquots, depending on the anticipated amount of parathion in the original sample, were placed in 250 ml. beakers and quickly evaporated at 80° to 90°C. to a moist residue. A gentle current of impinging air aided in the evaporation.

To the residue were added 10 ml. of 95 per cent ethanol, 10 ml. water, 2 ml. of 5 N HCl, and approximately 0.5 gram of powdered zinc. The resulting mixture, with the beaker covered by a watch glass, was maintained at 80° to 90°C. for 20 minutes. The reduction mixture was filtered at once through Sharkskin paper into a 100 ml. volumetric flask, and the filter cake washed with three 7-ml. portions of water. Exactly 1.0 ml. of 0.25 per cent sodium nitrite solution was added with shaking to the combined filtrates, followed after exactly 10 minutes by 1.0 ml. of 2.5 per cent ammonium sulfamate solution with shaking. After another 10 minutes, 2.0 ml. of a 1-per cent solution of N-(1-naphthyl)ethylenediamine dihydrochloride were added with shaking. The magenta color indicative of the parathion developed at this stage. After a 10-minute development period, 50 ml. of 95 per cent ethanol and 2 ml. of 3 N HCl were added, and the volume adjusted with water. If cloudy, the final mixture was extracted in the flask with two 5-ml. portions of petroleum ether (30° to 60° or 60° to 70°C.).

The transmittance of the final, optically clear solution was determined with a Beckman Model "B" spectrophotometer at a wave length of 555 mμ and a slit width of 0.2 to 0.4 mm. against a reagent blank.

The quantity of parathion was determined by comparing the observed transmittance with a standard curve prepared by analyzing known amounts of parathion by the same procedure.

Modifications

Removal of the greater portion of the solvent from the original aliquots was done with the Friedrichs condensers followed by the complete evaporation from the beakers on a hot-plate at a temperature of 80° to 90°C. No loss of parathion resulted from this procedure.

Most residue studies have been made on crops treated only with the insecticide under investigation; therefore, no information was available concerning the possible effect of another insecticide such as toxaphene on the analysis of parathion. However, tests showed no difference between recovery of parathion from solutions containing 5 to 10 mg. of toxaphene and those containing no toxaphene, nor did the toxaphene alone produce color measurable at 555 mμ.

Observations

This method has been widely adopted and is highly recommended by many workers. Sufficient work has been done to determine the most satisfactory conditions for usage, and no difficulties were encountered in its use.

APPENDIX B - - TABLES OF STATISTICAL ANALYSES

Table 22. - - Analysis of variance (random design) of toxaphene in mg. on treated filter papers water-washed with fog nozzles.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 8 | - - - | - - - |
| Washing time | 2 | 0.3934 | - - - |
| Error | 6 | 1.1965 | - - - |

Table 23. - - Analysis of variance (random design) of parathion in mg. on treated filter papers water-washed with fog nozzles.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 8 | - - - | - - - |
| Washing time | 2 | 0.0885 | 4.04 |
| Error | 6 | 0.0219 | - - - |

Table 24. - - Analysis of variance (random design) of toxaphene in mg. on treated filter papers water-washed by immersion.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 8 | - - - | - - - |
| Washing time | 2 | 0.6642 | 2.45 |
| Error | 6 | 0.2706 | - - - |

Table 25. - - Analysis of variance (random design) of parathion in mg. on treated filter papers water-washed by immersion.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 8 | - - - | - - - |
| Washing time | 2 | 0.0734 | 1.85 |
| Error | 6 | 0.0397 | - - - |

Table 26. - - Analysis of variance (split-plot) of weight loss, in per cent, of celery harvested 2 hours after spraying: 6 replicates, 4 washing treatments, and 3 storage times - - Winter, 1952-53.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 71 | - - - | - - - |
| Main plots | 23 | - - - | - - - |
| Replications | 5 | 2.987 | 1.71 |
| Washing treatments | 3 | 8.149 | 4.67 * |
| Error (a) | 15 | 1.745 | - - - |
| Sub-plots in main plots | 48 | - - - | - - - |
| Storage time | 2 | 36.207 | 143 ** |
| Washing treatment x storage time | 6 | 0.288 | 1.14 |
| Error (b) | 40 | 0.252 | - - - |

* Significant at the 0.05 level of probability.

** Significant at the 0.01 level of probability.

Table 27. - - Analysis of variance (split-plot) of weight loss, in per cent, of celery harvested 2 weeks after spraying: 5 replicates, 4 washing treatments, and 3 storage times - - Winter, 1952-53.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 59 | - - - | - - - |
| Main plots | 19 | - - - | - - - |
| Replications | 4 | 1.109 | - - - |
| Washing treatments | 3 | 0.661 | - - - |
| Error (a) | 12 | 1.534 | - - - |
| Sub-plots in main plots | 40 | - - - | - - - |
| Storage time | 2 | 17.138 | 138 ** |
| Washing treatment x storage time | 6 | 0.052 | - - - |
| Error (b) | 32 | 0.124 | - - - |

** Significant at the 0.01 level of probability.

Table 28. - - Analysis of variance (split-plot) of weight loss, in per cent, of green beans: 3 replicates, 4 washing treatments, and 3 storage times - - Spring, 1953.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 35 | - - - | - - - |
| Main plot | 11 | - - - | - - - |
| Washing treatments | 3 | 28.96 | 11.49 ** |
| Replications | 2 | 5.73 | 2.27 |
| Error (a) | 6 | 2.55 | - - - |
| Sub-plots in main plots | 24 | - - - | - - - |
| Storage time | 2 | 388.67 | 1050 ** |
| Washing treatment x storage time | 6 | 1.068 | 2.89 |
| C vs. D,E,G x storage time | 2 | 0.427 | 1.12 |
| D vs. E vs. G x storage time | 4 | 1.389 | 3.75 * |
| E vs. D, G x storage time | 2 | 2.201 | 5.95 * |
| E vs. G x storage time | 2 | 0.071 | - - - |
| E vs. D x storage time | 2 | 2.706 | 7.31 ** |
| D vs. G x storage time | 2 | 0.576 | 1.56 |
| Error (b) | 16 | 0.370 | - - - |

* Significant at the 0.05 level of probability.

** Significant at the 0.01 level of probability.

Table 29. - - Analysis of variance (randomized blocks) of toxaphene in mg. on green beans: 3 blocks, 5 washing treatments - - Spring, 1953.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 14 | - - - | - - - |
| Blocks | 2 | 18.231 | 5.33 ** |
| Washing treatments | 4 | 86.018 | 25.16 ** |
| Error | 8 | 3.418 | - - - |

** Significant at the 0.01 level of probability.

Table 30. - - Analysis of variance (randomized blocks) of parathion in mg. on green beans: 3 blocks, and 5 washing treatments - - Spring, 1953.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 14 | - - - | - - - |
| Blocks | 2 | 0.7447 | 36.86 ** |
| Washing treatments | 4 | 0.1412 | 6.99 * |
| Error | 8 | 0.0202 | - - - |

* Significant at the 0.05 level of probability.

** Significant at the 0.01 level of probability.

Table 31. - - Analysis of variance (randomized blocks) of toxaphene in mg. on green beans: 3 blocks and 5 washing treatments - - Fall, 1953.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 14 | - - - | - - - |
| Blocks | 2 | 556.02 | 19.58 ** |
| Washing treatments | 4 | 375.53 | 13.23 ** |
| Error | 8 | 28.39 | - - - |

** Significant at the 0.01 level of probability.

Table 32. - - Analysis of variance (randomized blocks) of parathion in mg. on green beans: 3 blocks and 5 washing treatments - - Fall, 1953.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 14 | - - - | - - - |
| Blocks | 2 | 5.715 | 53.93 ** |
| Washing treatments | 4 | 2.096 | 19.77 ** |
| Error | 8 | 0.106 | - - - |

** Significant at the 0.01 level of probability.

Table 33. - - Analysis of variance (split-plot) of weight loss, in per cent, of tomatoes: 6 replicates, 4 washing treatments, and 3 storage times - - Spring, 1953.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 71 | - - - | - - - |
| Main plots | 23 | - - - | - - - |
| Replications | 5 | 1.357 | 6.17 ** |
| Washing treatments | 3 | 0.336 | 1.53 |
| Error (a) | 15 | 0.220 | - - - |
| Sub-plots in main plots | 48 | - - - | - - - |
| Storage time | 2 | 20.445 | 222 ** |
| Washing treatment x storage time | 6 | 0.024 | - - - |
| Error (b) | 40 | 0.092 | - - - |

** Significant at the 0.01 level of probability.

Table 34. - - Analysis of variance (randomized blocks) of toxaphene in mg. on tomatoes: 5 blocks and 5 washing treatments - - Spring, 1953.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 24 | - - - | - - - |
| Blocks | 4 | 0.394 | 1.13 |
| Washing treatments | 4 | 0.6295 | 1.80 |
| Error | 16 | | |
| 2 missing plots | -2 | | |
| Error | 14 | 0.3499 | - - - |

Table 35. - - Analysis of variance (randomized blocks) of parathion in mg. on tomatoes: 6 blocks and 5 washing treatments - - Harvest I - - Spring, 1953.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 29 | - - - | - - - |
| Blocks | 5 | 0.00410 | 0.707 |
| Washing treatments | 4 | 0.00593 | 1.022 |
| Error | 20 | 0.00580 | - - - |

Table 36. - - Analysis of variance (randomized blocks) of parathion in mg. on tomatoes: 4 blocks and 5 washing treatments - - Harvest II - - Spring, 1953.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 19 | - - - | - - - |
| Blocks | 3 | 0.0369 | 16.04 ** |
| Washing treatments | 4 | 0.0025 | 1.09 |
| Error | 12 | 0.0023 | - - - |

** Significant at the 0.01 level of probability.

Table 37. - - Analysis of variance (randomized blocks) of toxaphene in mg. on mustard greens: 3 blocks and 5 washing treatments - - Fall, 1953.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 14 | - - - | - - - |
| Blocks | 2 | 895 | 1.31 |
| Washing treatments | 4 | 23,408 | 34.22 ** |
| Error | 8 | 684 | - - - |

** Significant at the 0.01 level of probability.

Table 38. - - Analysis of variance (randomized blocks) of parathion in mg. on mustard greens: 3 blocks and 5 washing treatments - - Fall, 1953.

| Source of variation | Degrees of freedom | Mean square | F value |
|---|---|---|---|
| Total | 14 | - - - | - - - |
| Blocks | 2 | 1.827 | 1.48 |
| Washing treatments | 4 | 266.639 | 216 ** |
| Error | 8 | 1.233 | - - - |

** Significant at the 0.01 level of probability.

## BIOGRAPHICAL NOTES

Buford Dale Thompson was born in Lake Wales, Florida on October 22, 1922. He attended public schools in that city and entered the University of Florida in 1939. He served as an officer in The United States Army from 1943 until 1946. He returned to the University of Florida in 1946, and received a Bachelor of Science in Agriculture, with honors, in February, 1948. He pursued graduate studies at the University of Florida and received a Master of Science in Agriculture in September, 1949.

He has been employed as Assistant Horticulturist with the Florida Agricultural Experiment Stations since 1948. His doctoral research has been conducted in cooperation with this organization.

He was married to Margaret Virginia Cody of Babson Park, Florida on August 20, 1944, and is the father of two children.

While at the University of Florida, he was elected to membership in Phi Sigma and Alpha Zeta, and is an associate member of The Society of The Sigma Xi.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of the committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council and was approved as partial fulfilment of the requirements for the degree of Doctor of Philosophy.

January 30, 1954

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